Non-spatial paired-associate memory function and anterior thalamic contribution

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Abbreviations

A24	area 24 of the anterior cingulate cortex
A25	area 25 of the medial prefrontal cortex
A29	granular area 29 of the retrosplenial cortex
A30	dysgranular area 30 of the retrosplenial cortex
A32	area 32 of the medial prefrontal cortex
A33	area 33 of the medial prefrontal cortex
ACC	anterior cingulate cortex
AD	anterodorsal thalamic nuclei
AI	agranular insular cortex
AM	anteromedial thalamic nuclei
ATN	anterior thalamic nuclei
ATN No Trace	ATN lesion odour-object retention test at 5-days
ATN Trace	ATN lesion odour-trace-object retention test at 5-days
Aud	primary auditory cortex
AUD	Alcohol use disorder
AV	anteroventral thalamic nuclei
CA1	Cornu Ammonis area 1 of the hippocampus
CA3	Cornu Ammonis area 3 of the hippocampus
Cg	cingulate cortex
CNQX	cyanquixaline
CO	cytochrome-oxidase
contra	contralateral
CREB	cAMP response element binding protein
DG	Dentate gyrus of the hippocampus
DG	dentate gyrus of the hippocampus
DGhilus	Hilus of the dentate gyrus of the hippocampus
DIEC	dorso-intermediate entorhinal cortex
DLB	Dementia with Lew Bodies
DLEC	dorsolateral entorhinal cortex
Egr-1/Zif268	early growth response gene 1/zinc-finger binding protein
ENR	enrichment
Fx	Fornix
GABA	γ-Aminobutyric acid
GAP-43	growth associated protein

HF	hippocampal formation
HPC	hippocampus
HPC	hippocampus
IEG	immediate early gene
IL	infralimbic cortex
ILN	intralaminar nuclei
ipsi	ipsilateral
LT	lateral thalamic aggregate
MB	mammillary bodies
MCC	midcingulate cortex
MD	mediodorsal thalamic nuclei
mEC	medial entorhinal cortex
MRI	magnetic resonance imaging
MS	multiple sclerosis
MT	posteromedial thalamic aggregate
MTT	mammillothalamic tract
NMDA	N-methyl-D-Aspartate
NT5	No Trace Group, 5-day retention test after criterion (or maximum of 43 sessions)
PC	parietal cortex
PCFx	postcommisural fornix
pCREB	phosphorylated cAMP response element binding protein
PDD	Parkinson's disease with dementia
PFC	prefrontal cortex
PH	parahippocampal cortex
Pir	piriform cortex
PPC	posterior parietal cortex
PRh	perirhinal cortex
PrL	prelimbic cortex
RAM	radial arm maze
Re	nucleus reuniens
RF	radiofrequency
Rh	rhomboid nucleus
ROI	region of interest
RSA	agranular (dysgranular) retrosplenial cortex
RSC	retrosplenial cortex

RSG	granular retrosplenial cortex
Sham No Trace	sham lesion odour-object retention test at 5-days
Sham Trace	sham lesion odour-trace-object retention test at 5-days
Sub	subiculum
T25	Trace Group, 25-day retention test after criterion (or maximum of 43 sessions)
T5	Trace Group, 5-day retention test after criterion (or maximum of 43 sessions)
T-New	Trace Group, novel paired association 25-days after criterion (or a maximum of 43 sessions)
tx	treatment
unilat	unilateral

Abstract

The anterior thalamic nuclei (ATN) are a central node in a complex 'hippocampal-diencephalic-cingulate' memory system. The integrity of the anterior thalamic nuclei is linked with severe amnesia in the Korsakoff's syndrome, localised thalamic infarcts and Alzheimer-type neurodegeneration. Firm conclusions regarding the contribution of individual diencephalic nuclei in clinical case are limited by uncertainty and variability in lesion extent and location. In rats, the ATN has consistently been linked to spatial memory function, but evidence of these nuclei additional contribution only extend to a couple of studies on memory for the temporal sequence of items. This thesis describes a novel non-spatial odour-object paired-associate task adapted from (Kesner, Hunsaker, & Gilbert, 2005). This task was designed to reveal neural activation following recall after acquisition and the influence of ATN lesions on temporal and non-temporal variations of the task. Experiment 1 found activation in the medial prefrontal cortex in intact rats following long-term consolidation of the non-spatial odour-trace-object paired-associate task. Experiment 2, also in intact rats, identified neural activation in the hippocampal CA1 as a critical region for recent recall of an odour-trace-object relative to the odour-object (non-temporal) paired-associate task. It was expected, for Experiment 3, that ATN lesions would produce severe behavioural deficits only in the 'temporal' version of odour-trace-object task. However, ATN lesions prevented acquisition of both the temporal and non-temporal non-spatial paired associate tasks. This novel behavioural findings were supported by pronounced downregulation of the immediate early gene Zif268 throughout key regions of the extended memory system. Sham rats trained on the odour-trace-object task in this final study, by showing increased CA1 Zif268 expression, replicated the earlier findings; this independent replication strengthens the association between dorsal CA1 and recall of a temporal non-spatial paired associate task.

The ATN influence episodic-like memory beyond ‘space’, and also time. This likely reflects their profound influence on cortical and hippocampal structures within the extended memory system.

Chapter 1.
Introduction

1.1 General Introduction

Central to cognition and emotion, memory defines the ability to retain and use information. Anterograde (inability to form new memories) and retrograde amnesia (the inability to retrieve past memories) refer to profound impairment in the presence of comparatively preserved cognitive functioning (Aggleton, 2014; Squire & Wixted, 2011). Clinical evidence has determined that diencephalic disruption or injury can result in severe anterograde amnesia (Aggleton & Brown, 1999; Carlesimo, Lombardi, & Caltagirone, 2011; Kopelman, 2015). Following damage to the diencephalon and medial temporal lobe (hippocampus; HPC), substantial overlap in amnesic syndrome suggests that the regions work interdependently to support memory function (Aggleton, 2008, 2014; Delay & Brion, 1969).

Damage to a number of nuclei and fibre tracts of the diencephalon are commonly involved in anterograde amnesia. Pathology of the anterior thalamic nuclei (ATN), mammillary bodies (MB) and the mammillothalamic tract (MTT), however, is most consistent (Aggleton, 2014). Chapter 2 provides details of regions within the diencephalon that are involved in clinical cases of amnesia. The strongest evidence for diencephalic involvement comes from ATN disruption in the alcoholic Korsakoff's syndrome, a severe amnesic syndrome resulting from prolonged thiamine deficiency (Harding, Halliday, Caine, & Kril, 2000; Kopelman, 2015). Relatively localised thalamic strokes to the MTT and less consistently with the ATN also produce severe memory impairments (Carlesimo et al., 2011). Neurodegenerative diseases have also implicated the diencephalon, providing instances of diencephalic injury and memory loss generally accompanied by a wider range of more complex symptomology (Braak & Braak, 1991a, 1991b; Houtchens et al., 2007; Rüb et al.,

2002). Pathology in clinical cases of Alzheimer's disease, Parkinson's disease and multiple sclerosis often extends across nuclei making it difficult to make firm conclusions on contribution of specific nuclei.

Aggleton and Brown (1999) and more recent review by Bubb, Kinnavane, and Aggleton (2017) discussed the extensive animal evidence that suggested episodic memory relies on an extended system of brain structures critical to memory function. Aggleton and Brown (1999), coined the term "extended hippocampal system" to describe the circuitry heavily involved in memory. They provide a description of a system originating from the HPC, comprising the ATN, MB, retrosplenial cortex, the prefrontal and anterior cingulate cortices, and the fornix and mammillothalamic fibre tracts. Lesions to the HPC as well as diencephalic nuclei and fibre tracts support the existence of this extended memory circuit (Aggleton, 2008; Aggleton, Amin, Jenkins, Pearce, & Robinson, 2011; Aggleton et al., 2010; Dalrymple-Alford et al., 2015). A more recent update of this approach defines the connections of a 'hippocampal-diencephalic-cingulate' network of memory (Bubb et al., 2017). This update shifts the focus from a hippocampus-centric system, to one that relies on the interconnected nature of the structures. Chapter 3 will discuss the structures and tracts, and their interconnectivity in this extensive memory system.

Clinical evidence reveals damage to the ATN, MTT and MB are the diencephalic structures most often associated with anterograde amnesia (Aggleton, 2014; Carlesimo et al., 2011; Harding et al., 2000; Kopelman, 2015). Chapter 4 expands on the notion of an extended memory system with extensive evidence from animal lesion studies. Animal models of diencephalic lesions help to increase lesion specificity to address the levels of variability encountered in clinical case studies. As ATN dysfunction is consistently implicated in human cases of diencephalic amnesia, the ATN is the focus of this thesis. Studies of ATN lesion rats

have reported significant spatial alternation and memory deficits in the T-maze, radial arm maze and water-maze (Aggleton, Hunt, Nagle, & Neave, 1996; Dalrymple-Alford et al., 2015; Perry, Mercer, Barnett, Lee, & Dalrymple-Alford, 2018; Sziklas & Petrides, 1999). There is evidence that ATN lesions also impair memory for non-spatial processing in temporal order tasks, which are also dependent on intact HPC functioning (Dumont & Aggleton, 2013; Wolff, Gibb, & Dalrymple-Alford, 2006).

Paired-associate tasks in rats provide an episodic-like memory task that can be used to examine the effects of brain injury on spatial and non-spatial paired-associate memory. Acquisition of these tasks has been shown to be dependent on the HPC only when individual stimuli are presented across spatial or temporal contexts (Gilbert & Kesner, 2002, 2003; Kesner et al., 2005). Variations of paired-associate tasks provide an opportunity to determine whether ATN lesions impair the ability to form associations across arbitrary non-spatial or temporal stimuli. Paired-associate tasks allow for memory acquisition, retention, and consolidation to be measured in a non-spatial temporal context following injury. A brief outline of covert pathology is provided (Chapter 4), identifying immediate early gene (IEG) expression following ATN lesions on structures within the 'hippocampal-diencephalic-cingulate' network (Bubb et al., 2017). Distal changes within this system provide an insight into the range of deficits commonly associated with injury to this nodal structure. For example, the retrosplenial cortex has been shown to be especially sensitive to ATN lesions. Lesions to the ATN consistently induce substantial hypoactivation of neural activity markers such as the IEGs, c-Fos and Zif268 in this region (Dumont, Amin, Poirier, Albasser, & Aggleton, 2012; Dupire et al., 2013; Jenkins, Dias, Amin, & Aggleton, 2002; Loukavenko, Wolff, Poirier, & Dalrymple-Alford, 2015; Perry et al., 2018). These distal effects following

lesions to the ATN are believed to add to the severity of memory deficits (Aggleton, 2008; Aggleton & Nelson, 2015).

1.2 Aims of the current study

To gain a broader understanding of memory deficits beyond hippocampal influence, clinical and animal research highlight structures within the diencephalon. Consistent evidence details significant memory deficits following injury to (or dysfunction in) regions such as the ATN within the diencephalon. It is also clear that ATN injury leads to functional alterations in the 'hippocampal-diencephalic-cingulate' network. These alterations, for example, IEG downregulation in the RSC seem likely to contribute to behavioural deficits. Non-human research of the ATN and memory is generally limited to spatial memory tasks. In these spatial memory tasks, the hippocampus and diencephalon appear to function interdependently. There is evidence that disruption to ATN function also impairs memory for information processing in non-spatial temporal order tasks. These tasks are also sensitive to HPC function. More evidence is needed, however, to determine exactly how the ATN are involved in memory beyond spatial processing and recall. Non-spatial paired-associate learning provides one approach to address this issue.

In experimental Chapters 5-7, acquisition of a novel non-spatial odour-object paired-associate task was tested in intact rats and ATN-lesion rats. In Chapter 5 the non-spatial task was tested across a temporal lag (odour-trace-object) in intact rats. The aim of this study was to compare recent (at 5 days post-acquisition) and remote recall (at 25 days) and examine neural activation following consolidation of this paired-associate task. Chapter 6 compared IEG regulation in intact rats following acquisition and recent recall (at 5 days only) of No Trace versus Trace variations of the odour-object paired-associate task. Zif268 neural

activation was measured following retention to compared key regions involved in recall of both conditions. Chapter 7 compared acquisition of the odour-object and odour-trace object paired-associate tasks in Sham rats and ATN lesion rats. This study examined whether ATN lesions impaired acquisition on either variant of this task. It is possible that ATN lesions would leave performance intact only when no trace (i.e. no temporal) component was used; if so, then the odour-object (No Trace) paired-associate task could be used to assess consolidation effects after ATN lesions in a future study. IEG neural activation was also measured in both lesion surgery groups following acquisition and recall of the No Trace and Trace conditions of the paired-associate task.

1.3 Outline of thesis

The following chapter provides an outline for the involvement of the anterior thalamic nuclei in clinical diencephalic amnesia through the examination of clinical case studies. Chapter three introduces the concept of an extended 'hippocampal-diencephalic-cingulate' memory network and briefly describes the structures within the circuit and their interconnectivity. The next chapter (Chapter 4) examines ATN lesion models of memory deficits and provides experimental evidence for the involvement of these nuclei in spatial memory function. The use of paired-associate tasks is also investigated in this chapter. The latter examines ATN lesions as well as lesions to the wider circuit of memory related structures. Chapter four also discusses the use of IEG changes to determine neural activation as well as the distal changes following ATN lesions. Three subsequent empirical chapters follow: Chapter 5) IEG response of a novel non-spatial paired-associate memory task: Recent vs remote memory; Chapter 6) A novel non-spatial paired-associate memory task: IEG correlates of the temporal context; and Chapter 7) ATN Lesions: No-Trace/Trace Odour-Object Association. A general

discussion of the experimental and theoretical relevance of this thesis, as well as limitations and suggestions for future directions, is provided in Chapter eight.

Chapter 2.

Clinical Diencephalic Amnesia

Brain injury and neuropathology in the diencephalon is associated with clinical amnesia. In the past the vast majority of research into memory loss has been hippocampal-centric. Reviews of the literature, however, point to a neural system critical for episodic memory in the diencephalon and its connections with neocortical structures (Aggleton, 2014). The following section summarises evidence for diencephalic system-wide involvement in memory following Wernicke-Korsakoff's syndrome, lacunar stroke, neurodegenerative disease and acute brain injury.

2.1 Wernicke-Korsakoff's syndrome

Wernicke's encephalopathy is an acute neuropsychiatric reaction to thiamine (vitamin B1) deficiency (Kopelman, Thomson, Guerrini, & Marshall, 2009). Key characteristics of Wernicke's encephalopathy are confusion, ataxia (lack of coordination), nystagmus (repetitive, involuntary eye movement) and opthalmoplegia (paralysis of the muscles within and/or surrounding the eye; Wernicke (1881). Thiamine is an essential coenzyme found in many metabolic pathways in the brain, including carbohydrate and lipid metabolism, and in the production of amino acids and glucose-derived neurotransmitters (i.e. GABA; Sechi and Serra (2007). Midline brain pathology generally associated with Wernicke's encephalopathy is most common in individuals who abuse alcohol. However, similar pathology is sometimes found when nutrient absorption is compromised (i.e., AIDs, anorexia nervosa with purging and medications to treat peptic ulcers; Sechi and Serra (2007).

Korsakoff's syndrome is characterised by severe memory deficits with otherwise generally intact cognitive functioning (Kopelman et al., 2009). Korsakoff's syndrome

usually, but not always emerges from an episode of Wernicke's encephalopathy and is also a result of thiamine deficiency. The striking loss of memory function is the critical symptom to distinguish Korsakoff's syndrome from Wernicke's encephalopathy. Memory deficits are generally associated with neuronal loss, micro-haemorrhages and gliosis in the paraventricular and peri-aqueductal grey matter (Victor, Adams, & Collins, 1971). Korsakoff's patients have severe memory loss, but retain implicit learning ability (e.g. are still able to learn new motor skills), so there is close resemblance to patients with amnesia after medial temporal lobe injury (Squire & Wixted, 2011).

Neuropathology throughout regions in the diencephalon has commonly been reported in both Wernicke's encephalopathy and Korsakoff's syndrome. As there is an overlap of the two conditions, it has proven difficult to confirm the critical site of pathology causing amnesia. Early research has been criticised for the lack of sufficient neuropsychological evidence to support post-mortem neuropathology of the patients the mammillary bodies (Kopelman, 2015). For some time, the mediodorsal thalamus were regarded critical sites of pathology for distinguishing Wernicke's from Korsakoff's syndrome (Victor et al., 1971). Following more extensive neuropsychological testing and post-mortem examination of brain tissue, W. G. Mair, Warrington, and Weiskrantz (1979) and Mayes, Meudell, Mann, and Pickering (1988) suggested that the mammillothalamic tract (MTT), mammillary bodies (MB) and anterior thalamus were critical sites of pathology for the manifestation of amnesic Korsakoff's syndrome. Their conclusions were supported by Harding et al. (2000) who provided evidence that neuronal loss in the mammillary bodies and mediodorsal thalamus are present in both Wernicke's syndrome and Korsakoff's syndrome, but more degeneration in the anterior thalamus distinguished the memory impaired Korsakoff's patients (Figure 2.1).

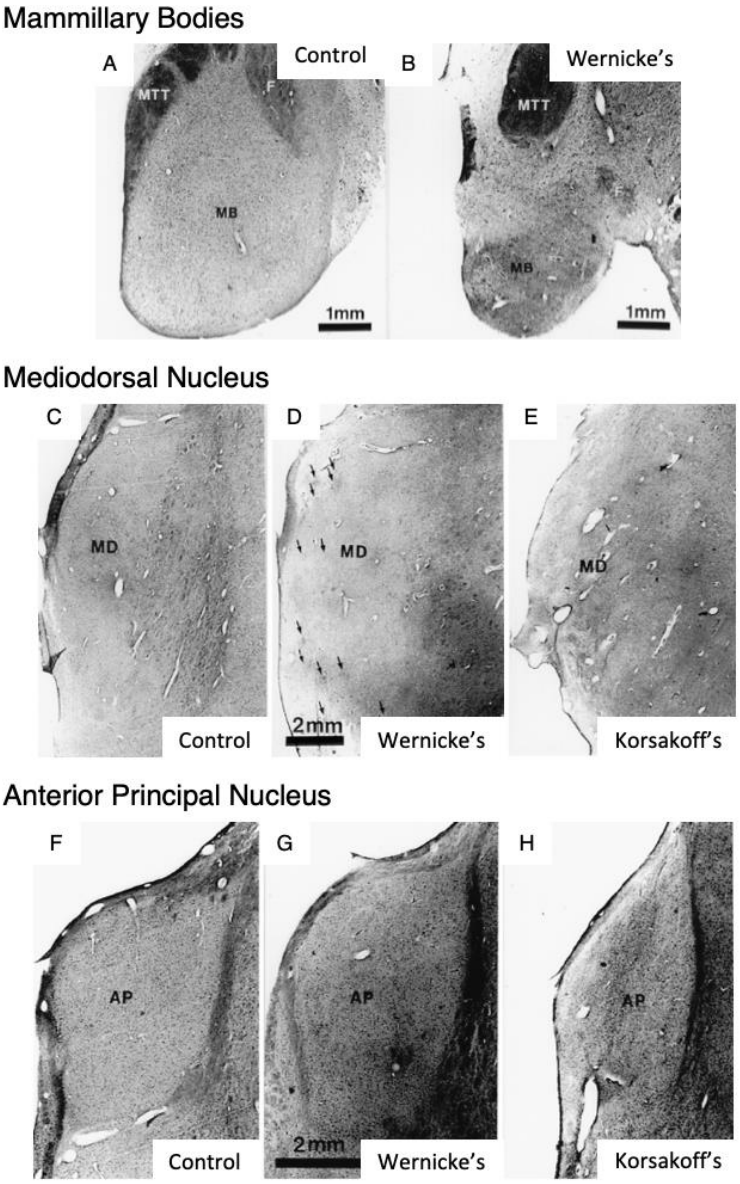

Figure 2.1. Photomicrographs comparing the mammillary body, mediodorsal and anterior thalamus of healthy controls and patients presenting both Wernicke's and Korsakoff's syndromes. MB comparing a healthy control (A) and an alcoholic with chronic Wernicke's syndrome (B); of note is the darkened appearance of the shrunken MD in the Wernicke's patient. MD comparing a healthy control (C), a patient with Wernicke's syndrome (D), a patient with Korsakoff's syndrome (E); of note is the vascular changes in both Wernicke's and Korsakoff's syndrome, arrows showing petechial haemorrhage. Anterior thalamus (commonly known as the anterior principal nucleus (AP) in humans) comparing a healthy control (F), a patient with Wernicke's syndrome (G), a patient with Korsakoff's syndrome (H); of note is the shrunken appearance of the AP in the Korsakoff's patient. Adapted from Harding et al. (2000).

Neural imaging studies also provide evidence for the involvement of the thalamus and MB in amnesic Korsakoff's syndrome (Kopelman, 2015). Early imaging studies showed a varied degree of cortical atrophy, with the frontal lobes being implicated (R. R. Jacobson,

Acker, & Lishman, 1990; Shimamura, Jernigan, & Squire, 1988). Later work comparing grey and white matter has shown more severe atrophy in the thalamic nuclei, MB, and corpus collosum in Korsakoff's patients than non-amnesic alcoholics, once again highlighting the thalamic and mammillary regions as critical sites in the presence of diencephalic amnesia (Pitel et al., 2012). A more recent imaging study was designed to determine the underlying mechanisms of thalamic alterations in alcohol use disorder (AUD) and Korsakoff's syndrome (Segobin et al., 2019). Using probabilistic tractography derived from diffusion tensor imaging, they defined thalamic segmentations according to circuit wide connectivity in healthy controls. Comparison of these healthy controls with patients having a history of chronic and excessive alcohol consumption indicated damage to the mediodorsal and anterior thalamus. While damage to the mediodorsal thalamus is similar in both AUD and Korsakoff's syndrome, atrophy of the anterior thalamus appeared to be specific to Korsakoff's syndrome. Comparison of thalamic injury across progressive stages from AUD to Korsakoff's syndrome suggests that the anterior thalamus has the strongest potential as a neuroimaging marker for identifying Korsakoff's syndrome and other disorders linked to thalamic amnesia. Other thalamic structures, including the MD, may have a less critical contribution.

2.2 Thalamic stroke

Brain injury caused by stroke is due to a disruption to the blood supply. This can cause a wide variety of symptoms including vertical gaze palsy, executive function deficits, inattention and confusion as well as amnesia (Li et al., 2018). Reasons for stroke include ischemia (lack of blood flow), clot (thrombosis or arterial embolism), or haemorrhage (leaking of blood into the tissue). Infarctions of the thalamus are common, and those affecting the anterior region of the thalamus (**A**, Figure 2.2) accounts for around 12% of all such cases (Carrera & Bogousslavsky, 2006). Clinical features of anterior thalamic infarcts present with

a range of neuropsychological deficits, from impaired learning and memory to hemispheric dependent deficits, left dependent loss of language (Carrera, Michel, & Bogousslavsky, 2004) and right dependent visuospatial deficits (Ghika-Schmid & Bogousslavsky, 2000). Memory impairment generally results from damage to anterior and medial thalamic nuclei, as well as the internal medullary lamina, the fornix (descending to the MB) and the MTT (Schmahmann, 2003). Ghika-Schmid and Bogousslavsky (2000) found that all patients in their study with anterograde memory impairment had lesions isolated to the anterior thalamus region, including the MTT and internal medullary lamina. People with anteromedian infarctions (combing posterior sections of the anterior territory, and the anterior region of the paramedian territory of the thalamus) also commonly display neuropsychological disturbances, with anterograde amnesia a common symptom. Carrera et al. (2004) reported that 89% of patients with anteromedian localisation (**B**, Figure 2.2) presented with amnesic symptoms; 75% unilateral left, 100% unilateral right and bilateral lesions.

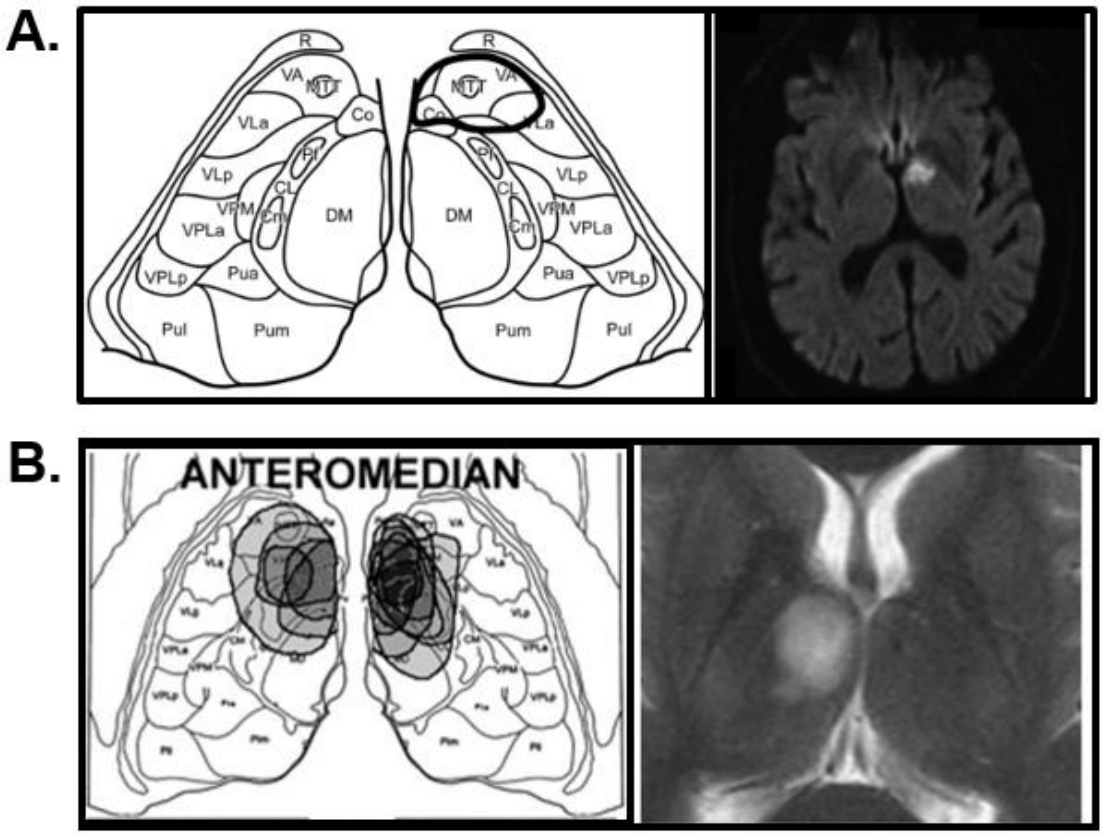

Figure 2.2. **A.** Anterior infarct segmentation (left) template thalamus with representation of the anterior regions implicated; diffusion weighted MRI (right) example infarct encompassing the mammillothalamic tract and anterior thalamus. **B.** Anteromedian infarct (Carrera et al., 2004). Left: template of the anteromedian thalamic territories. Right: Example infarct (left hemisphere in image) of a combination of anterior and medial thalamic territories. Adapted from Carrera and Bogousslavsky (2006).

Recent in vivo neuroimaging studies provide relatively consistent evidence for localising the key thalamic pathology associated with an amnesic syndrome. Carlesimo et al. (2011) reviewed papers using neuroimaging and neuropsychological techniques published between 1983 and 2009 for patients who had presented with thalamic ischemic stroke. They concluded that patients with vascular thalamic amnesia, like those with medial temporal lobe damage, displayed intact short-term memory and implicit memory, but showed deficits in declarative anterograde memory and, less consistently, retrograde memory. Van Der Werf et al. (2003), in cases of thalamic infarction, presented evidence of a strong association between MTT damage and severe amnesia. In this study, thalamic injury was determined through MRI

imaging with extensive neuropsychological measures. In this lesion-overlap study, a compilation of images of the lesions of patients with amnesic patients were compared with lesions from patients with no memory deficits. This overlap analysis provided a clear structure-function relationship to determine the MTT (Figure 2.3) as a critical structure in memory dysfunction.

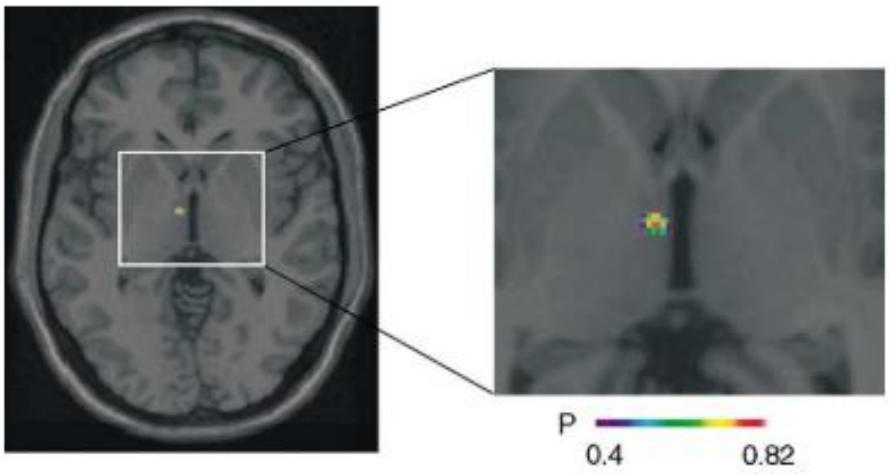

Figure 2.3. Images showing mammillothalamic tract lesion overlap in patients with amnesic syndrome. The figure shows and overlap of lesions from patients who presented with amnesic syndrome. Image is thresholded between p = 0.4 and 0.82 (Van Der Werf et al., 2003).

More recently, Danet et al. (2015) compared MRI and neuropsychological assessments of patients with left thalamic stroke to healthy controls. Following manual segmentation, lesions were automatically localised to a thalamic atlas to provide a much clearer image of lesion distribution than previous neuroimaging studies (Figure 2.4). Consistent with the evidence above, patients with mammillothalamic tract damage presented with more severe memory impairment than patients with intact mammillothalamic tracts. These lesions produced similar severe memory loss as seen in Korsakoff's patients with anterior thalamic lesions (Harding et al., 2000). Notably, however, there was also evidence that isolated damage to the mediodorsal thalamus results in moderate memory impairment. Unexpectedly, only one patient in this study was found to have obvious ATN damage. This

study highlights the notion that thalamic lesions are seldom specifically localised and can associate with multiple disconnections.

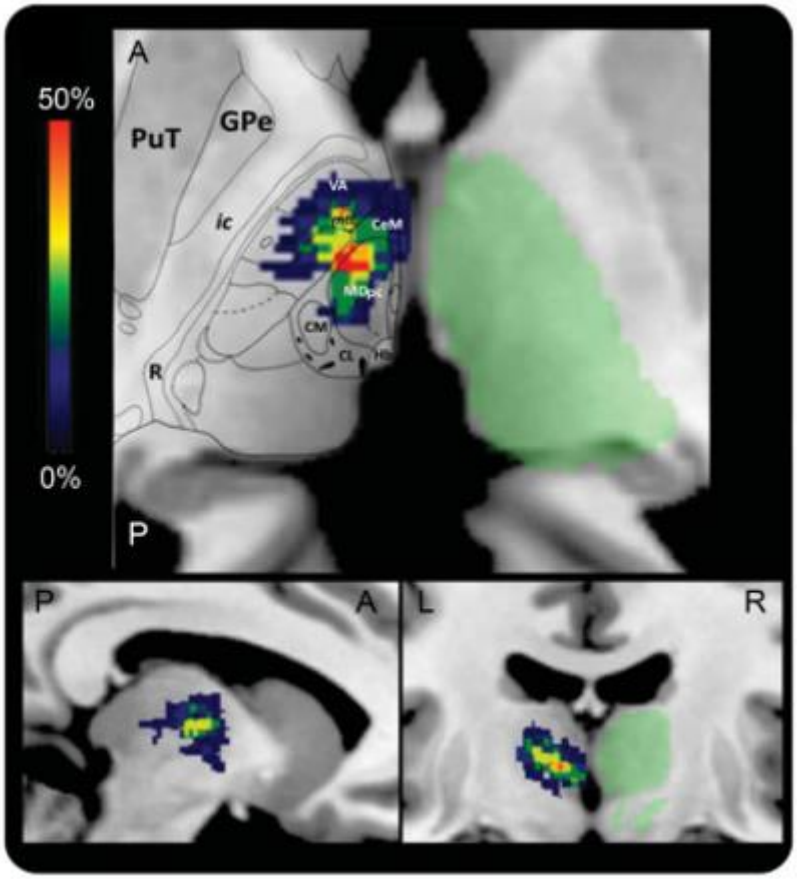

Figure 2.4. Example of a segmented localised thalamic lesion. Overlap of lesions across patients (n=12) in an axial view. Green mask and atlas template of the thalamus provided for extra detail (Danet et al., 2015). A=Anterior; CeM = central medial; CL = central lateral; CM = centromedian; GPe = external globus pallidus; Hb = habenula; ic = internal capsule; MDpc = parvocellular part of the mediodorsal nucleus; mtt = mammillothalamic tract; PuT = putamen; R = reticular nucleus; VA = ventral-anterior; P=posterior

Both Korsakoff's syndrome and thalamic stroke provide strong evidence that structures within the medial diencephalon support memory function. Debate continues on the critical site of damage causing memory deficits. As outlined above, damage to the anterior thalamus, MB, MTT and the mediodorsal thalamus have been consistently implicated in clinical studies of thalamic amnesia. Kopelman (2015) provided a review of similarities between Korsakoff's syndrome and thalamic amnesia in which there are deficits in

recollective memory (see Table 2.1). While Korsakoff's syndrome also shows evidence of deficits in recognition and temporal context memory, only a third of patients with thalamic stroke present with these deficits (Kopelman, 2015). The similarities and distinctions between these conditions, highlights the significance of determining the contribution to memory of the different structures that have been implicated.

Table 2.1. Comparison of the main neuropsychological and neuroimaging (red boxes) findings in patients with Korsakoff's syndrome and thalamic stroke (infarction) (Kopelman, 2015).

Neuropsychology	Korsakoff patients	Thalamic infarct patients
Primary/short-term memory		
Traditional measures	Intact	Intact
'Working memory' measures	All components impaired but no worse than non-Korsakoff alcoholics	Not tested
Anterograde episodic memory		
Recall/recollection	Severely impaired	Severely impaired
Recognition/familiarity	Impaired, but can be compensated by prolonged exposure times	Spared in some studies; impaired in others
Context memory	Temporal context memory always affected; spatial more variable	Recollection deficit implies impairment, but less specifically studied than in Korsakoff's
Retrograde amnesia	Invariably present and usually prolonged (20–25 years) with a temporal gradient	Present in approximately one-third of patients, sometimes 'extensive', sometimes brief (1–10 years)
Confabulation	Seen in acute confusional phase (Wernicke), but spontaneous confabulation in chronic phase only if extensive frontal or ventro-medial frontal damage. Mechanism controversial	Seldom if ever reported
Neuroimaging		
MRI		
Principal findings	Atrophy in thalami, mammillary bodies and frontal cortex	Critical lesions have been reported in the anterior nuclei, mammillo-thalamic tract and medio-dorsal nuclei. May be unilateral or bilateral
Associated findings	Atrophy in hippocampi (varying severity), amygdala, and cerebellar vermis	
PET	Reduced metabolism in the thalami, mammillary bodies, basal forebrain/orbito-frontal cortex, retrosplenium/precuneus. Also implicated: middle cingulate gyrus, superior frontal gyrus, temporal and occipital cortex	Few studies: thalamic and retrosplenial hypometabolism implicated. Widespread unilateral cortical hypometabolism has also been reported

2.3 Neurodegenerative disease

Neurodegenerative disorders that affect the thalamus include multiple sclerosis (MS), Lewy Bodies Dementia (DLB; Parkinson's disease with dementia, PDD), and Alzheimer's disease. MS is a progressive disease where central nervous system atrophy occurs involving both axonal and neuronal loss (Houtchens et al., 2007; Rocca et al., 2015). The anterior thalamus has been implicated in MS. For example, a case study provided evidence that left anterior thalamic regional damage disrupts the default mode network, resulting in cognitive disruption (D. T. Jones, Mateen, Lucchinetti, Jack, & Welker, 2011). MRI of patients with MS has shown that one of the strongest predictor of impaired cognitive function is thalamic atrophy

(Houtchens et al., 2007; Sumowski et al., 2018). Atrophy of hippocampal subregions (especially CA1) has also been shown as a predictor of encoding and retrieval deficits in MS patients (Sicotte et al., 2008).

PDD presents with many clinical symptoms, including deficits in memory, attention, visual perception and cognitive fluctuations (Emre, 2003; Gratwicke, Jahanshahi, & Foltynie, 2015). Autopsy examinations provide evidence that clinically diagnosed PDD patients have Lewy body pathology within the thalamus, especially the limbic thalamic nuclei (Rüb et al., 2002). Intralaminar nuclei pathology correlates with progression to dementia, and may be a correlate with memory impairment due to its projections to prefrontal and anterior cingulate cortices (Halliday, 2009).

The severe episodic memory deficit that characterises Alzheimer's disease has been linked to a 'hippocampal-diencephalic-cingulate' (Bubb et al., 2017) network (Aggleton, Pralus, Nelson, & Hornberger, 2016). These authors emphasise the need to think outside of the medial temporal lobe for memory deficits associated with Alzheimer's disease. Traditionally of course, memory impairments in Alzheimer's disease have been primarily attributed to hippocampal dysfunction. However, changes in early Alzheimer's disease are also found in the anterior thalamus and retrosplenial cortex (Braak & Braak, 1991a, 1991b; Delacourte et al., 1999). In support of this, de Jong et al. (2008) determined that profound atrophy in the putamen and thalamus in probable Alzheimer's disease patients, with additional global decrease in grey matter and hippocampal volumes, correlated with impaired memory and cognitive performance.

2.4 Traumatic brain injury

Traumatic brain injury with damage to deeper brain structures (i.e. thalamus) are rare. There are two well known cases. Patient B.J developed severe amnesia (anterograde and retrograde) when a snooker cue penetrated his left nostril (Dusoir, Kapur, Byrnes, McKinstry, & Hoare, 1990). Patient N.A developed severe amnesia for primarily verbal material when a fencing foil penetrated his right nostril (Squire, Amaral, Zola-Morgan, Kritchevsky, & Press, 1989). These cases provide important information regarding the time course for the injuries, but multiple diencephalic regions were injured. Patient B.J presented with damage to the hypothalamus and MB (presumably also the MTT). However, the subsequent recovery of patient B.J's memory function, is likely to be due to the sparing of damage to the nuclei within the thalamus (Dusoir et al., 1990). MRI showed that patient N.A. had prominent lesions in the left thalamus (including the intralaminar nuclei, ventral MD, the ventral lateral, and ventral anterior nuclei), as well as complete lesions to the MB and likely damage to both the MTT and postcommisural fornix (Squire et al., 1989). Damage to multiple structures following these two penetrating injuries makes attributing amnesic syndrome to a single structure difficult.

Evidence for the involvement of the MB has come from damage as a consequence of the removal of colloid cysts. Colloid cysts are benign tumours that develop on the third ventricle adjacent to the fornix. MB atrophy as a subsequent result of fornix injury is associated with the surgical removal of colloid cysts (Tsivilis et al., 2008). Tsivilis et al. (2008) determined that fornix volume alone was not consistently associated with memory deficits. MB volume, however, correlated with 13 out of 14 episodic memory tests of recall, but not recognition memory. In addition, patients with the smallest MB volume presented with the greatest episodic memory impairment.

2.5 Concluding remarks

The clinical evidence for diencephalic amnesia presented above supports the idea of a network of memory-related regions in which the anterior thalamus and the mammillothalamic tract are key structures. Evidence of amnesia following injury beyond the hippocampus indicates that these structures, along with the mammillary bodies, and perhaps also the mediodorsal thalamus contribute to episodic memory function. The following chapter will outline evidence for an extended memory system, briefly describing its structures and their connectivity. Differences in severity and additional brain injury in even the most localised clinical cases prevents conclusions regarding a structures' specific involvement in memory. Thus, highlights the value of animal models to provide better lesion specificity and experimental control to determine the impact these diencephalic structures have on memory.

Chapter 3.

The ‘hippocampal-diencephalic-cingulate’ network

This chapter examines the brain regions involved in episodic memory, which set the context of the current this study. A general overview of the ‘hippocampal-diencephalic-cingulate’ network will be described, with descriptions of key structural components and their connectivity within this network. A diagrammatic representation of these structures and connectivity are shown in Figure 3.1. There is particular focus on the anterior thalamic nuclei (ATN) due to its diverse neural connections, which may explain the relevance of the ATN in diencephalic amnesia (Chapter 2).

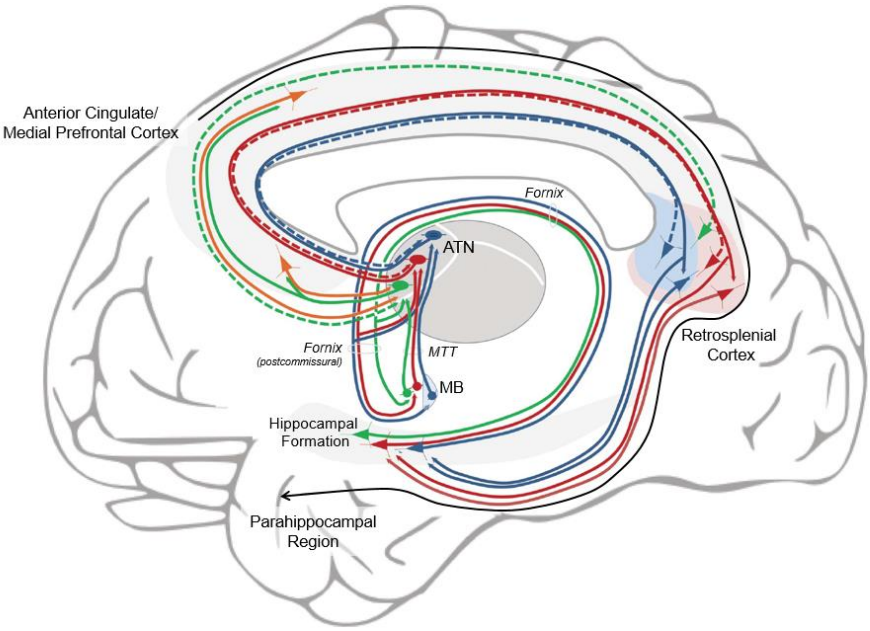

Figure 3.1. Schematic diagram representing the major components of the extended memory system discussed in Chapter 3.Coloured lines detail connectivity for each of the anterior thalamic nuclei: blue = anterodorsal thalamic nuclei; red = anteroventral thalamic nuclei; green = anteromedial thalamic nuclei. Adapted from Child and Benarroch (2013).

3.1 Progression from Papez to the 'hippocampal-diencephalic-cingulate' network

The circuit described by Papez (1937) implicated an interconnected set of structures (**A**, Figure 3.2), including the parahippocampal region, hippocampus (HPC), mammillary bodies (MB), and cingulate cortices involved in the control of emotion. Delay and Brion (1969) developed this further, but as a circuit responsible for memory given evidence of amnesia following damage or injury to these regions. The connectivity of this circuit was re-examined by Aggleton and Brown (1999) who proposed an "extended hippocampal system" implicated in the encoding and recall of episodic memory (**B**, Figure 3.2). Based on extensive clinical and experimental animal work, this system centres on the hippocampal formation and its fornix and postcommisural fornix (PCFx) connections with the anterior thalamus and the MB, as well as connections of the ATN with the prefrontal and retrosplenial cortices and the hippocampus (Aggleton & Brown, 1999). This reformulation differs in that it places emphasis on the efferents from the HPC via the fornix to the diencephalon.

A more recent anatomical description by Bubb et al. (2017) added developments that expand on the networks outlined above (Aggleton & Brown, 1999; Delay & Brion, 1969). To emphasise this interactive system, they defined a 'hippocampal-diencephalic-cingulate network' implicated in memory. Greater emphasis was now placed on the multiple parallel and reciprocal interactions of the various structures within the network. Their review identified key drawbacks of the previously proposed circuits, especially the 'serial' connectivity of regions defined by Delay and Brion's (1969) 'hippocampal-cortical-hippocampal network'. Providing an anatomical guide (**C**, Figure 3.2), this review highlights connections of the hippocampus and parahippocampal region with the MB, ATN and the cingulate gyrus. The following sections will provide an overview of the regions and connections outlined in **C**, Figure 3.2.

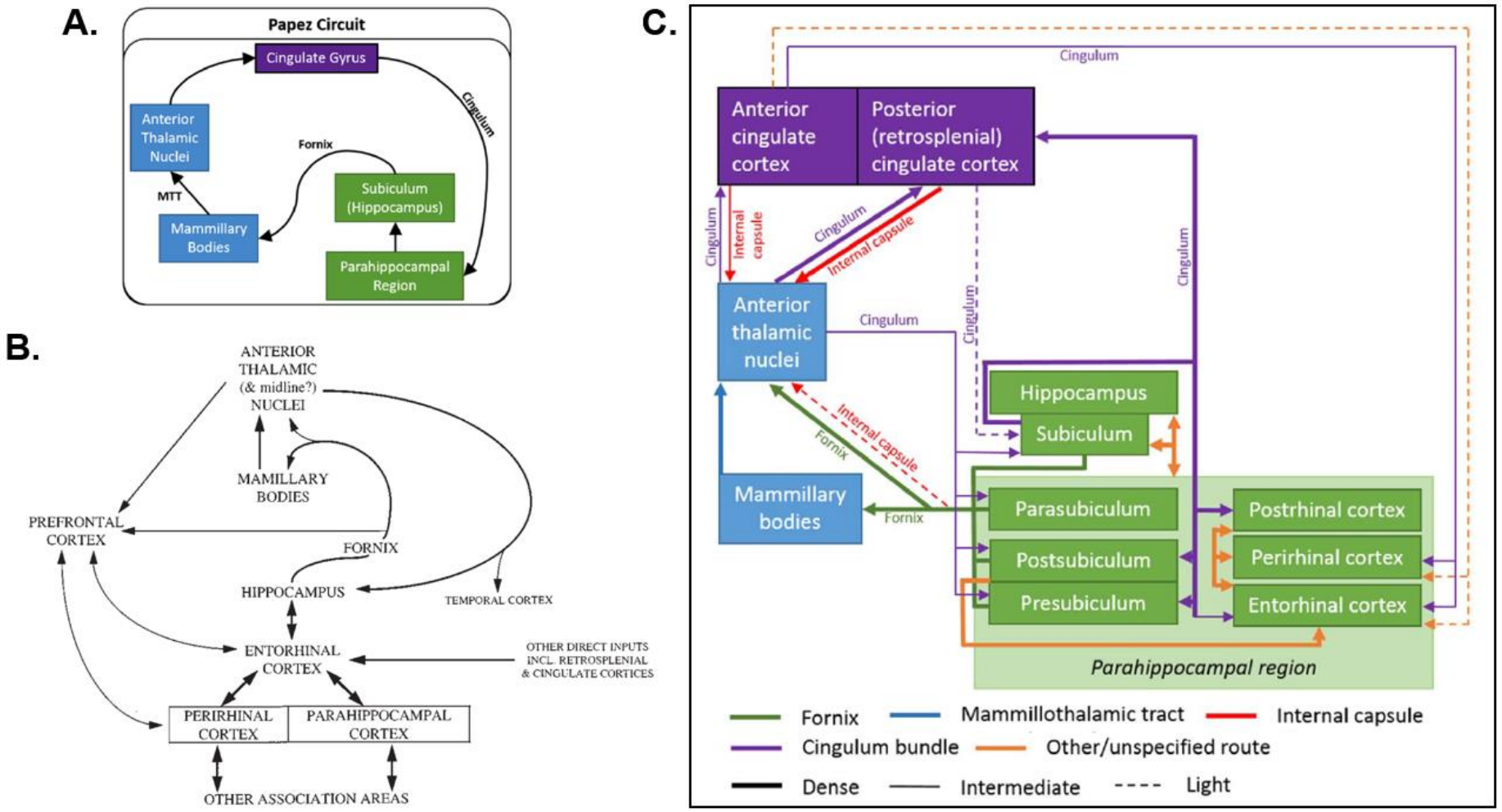

Figure 3.2. Progression of the extended memory system from Papez (**A**) and the extended hippocampal system (**B**) to the 'hippocampal-diencephalic-cingulate' network (**C**). **A.** traditional depiction of Papez circuit (1937), arrows show direction of connections. **B**. The extended hippocampal system outlined by Aggleton and Brown (1999, 2006) showing the main connections between regions in the system. **C**. Schematic of the 'hippocampal-diencephalic-cingulate' network outlined by Bubb et al., (2017) showing the main/direct interconnections between the sites in the circuit (some connectivity not shown). Thickness of the lines reflects the strength of the connections. **A.** and **C.** adapted from Bubb et al. (2017); **B.** From Aggleton and Brown (1999) Abbreviations: ATN = anterior thalamic nuclei; MB = mammillary bodies; MTT = mammillothalamic tract.

3.2 Structures within the 'hippocampal-diencephalic-cingulate' network

The hippocampus and fornix

The hippocampus, located in the medial temporal lobe bordering the rostral brain stem, is a major component of the limbic system. There is consensus that the hippocampus is critically involved in spatial memory and contextual learning and episodic memory more generally, at least in humans (Squire & Wixted, 2011). The hippocampus consists of the CA1-4 subfield, the dentate gyrus and is described as the hippocampal formation (HF) with the inclusion of the subiculum (Bubb et al., 2017; Squire, Stark, & Clark, 2004). The rat hippocampus has a dorsal-ventral division, corresponding to human anterior-posterior. The fornix, a C-shaped white matter bundle, carries fibres to and from the hippocampus of both hemispheres that come together anteriorly in the midline forming the fornix. Efferent HF fibres then descend as the pre- and postcommisural fornix (Vann, Erichsen, O'Mara, & Aggleton, 2011).

The mammillary bodies and mammillothalamic tract

The mammillary bodies (MB) have long been considered an important structure in learning and memory (Delay & Brion, 1969). They are located on the posterior margin of the hypothalamus in the diencephalon. The MB consist of of two nuclei (Vann & Aggleton, 2004). The medial mammillary nuclei are larger and composed of a group of up five subnuclei, which varies with species. The lateral nuclei are much smaller but contain the largest cells within the mammillary bodies (Vann & Aggleton, 2004). Efferents from the MB form the mammillothalamic tract (MTT). This important fibre tract is unique by having only unidirectional projections that arise from the medial and lateral mammillary nuclei and which terminate in the anterior thalamus (Vann, Saunders, & Aggleton, 2007)

The anterior thalamic nuclei

Among thalamic nuclei, the ATN are considered to be key components of the extended memory system that collectively support episodic memory (Aggleton, 2008; Child & Benarroch, 2013). The ATN are located in the anterior medial portion of the thalamus, separated from other thalamic nuclei by the anterior internal medullary lamina (S. Jacobson & Marcus, 2011). The ATN consists of three subnuclei: the anterodorsal (AD), anteroventral (AV), and anteromedial (AM) nuclei (**A**, Figure 3.3). In the rat, these three subnuclei have a distinctive cytoarchitecture when observed after a Nissl stain. The AD is the smallest nuclei and has large, dense cells under Nissl stain. The AV, the largest of the three nuclei, is composed of more lightly stained cells that are less densely compact than the AD. The AM displays larger cells that are more palely stained with Nissl. Although the AV and AM are comparable in size and their borders relatively easily defined in the rat (Price, 1995), this is not the case in humans (Bentivoglio, Kultas-Ilinsky, & Ilinsky, 1993). Within the proposed 'hippocampal-diencephalic-cingulate' system, each of the anterior thalamic subnuclei has its own distinct pattern of connectivity (B, Figure 3.3; described in further detail below).

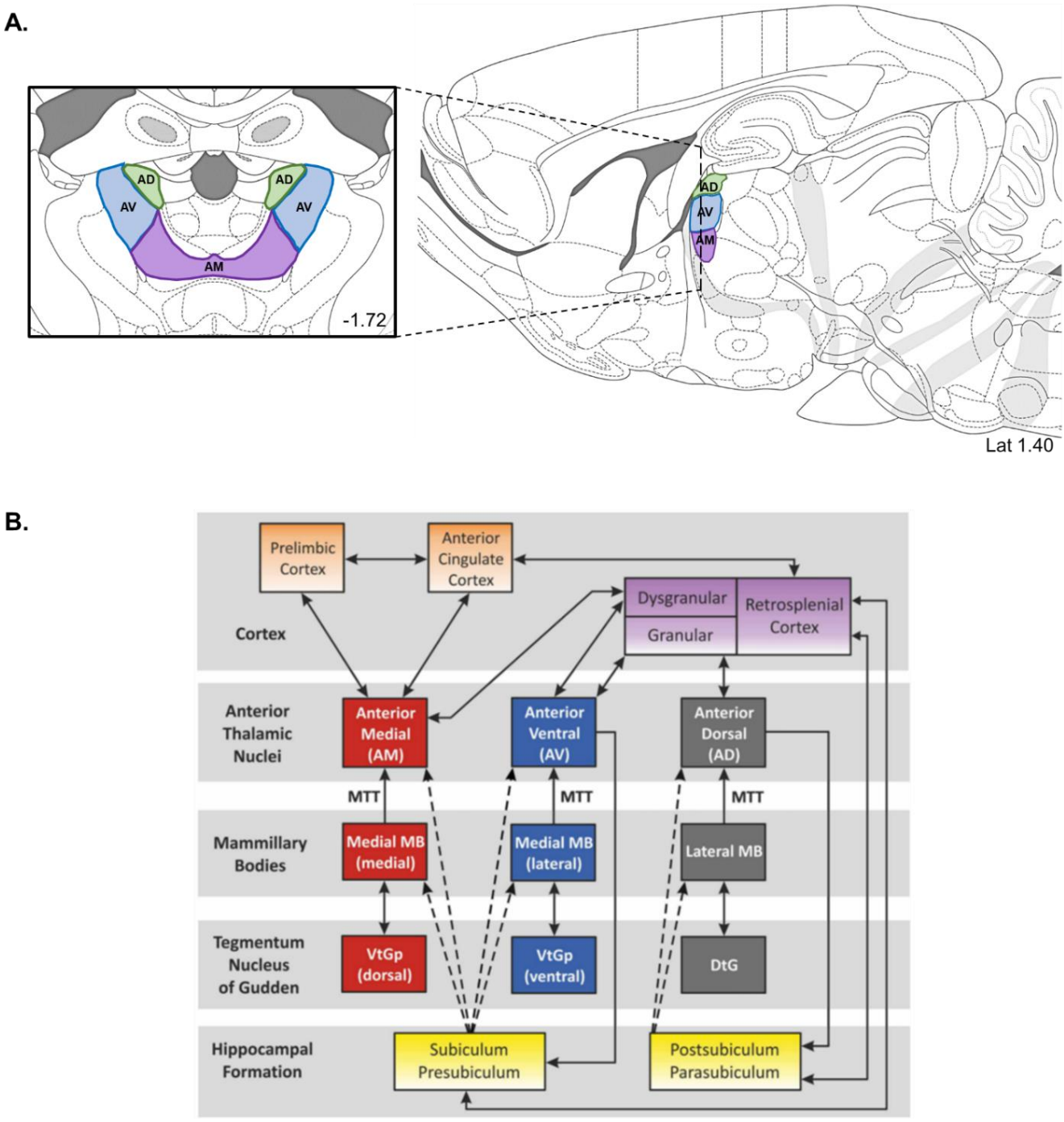

Figure 3.3. **A.** Anterior thalamic subnuclei divisions in the rat brain. Section plates adapted from Paxinos and Watson (2014). AD = anterodorsal thalamic nuclei; AM = anteromedial thalamic nuclei; AV = anteroventral thalamic nuclei. Atlas number (in mm), relative to Bregma; Lat = denotes the laterality of section in mm. **B.** Anterior thalamic connectivity in the extended memory system. Note the additional anterior cortical connections of the AM as well as the RSC. **A** & **B** adapted from Jankowski et al. (2013).

The cingulate cortex and cingulum bundle

The cingulate cortex, located dorsal to the corpus callosum, is a combination of large cortical regions occupying space from the anterior to the posterior poles of the brain. There are three distinct cingulate regions: anterior, mid, and posterior (the latter is retrosplenial in the rat). In this thesis, I will be referring to the cingulate regions as outlined by Vogt and Paxinos (2014). A visual comparison of the previous and current terminology for the cingulate regions is supplied in Figure 3.4. Following their examination of the cingulate regions, Vogt and Paxinos (2014) concluded that the rat cingulate cortex is comprised of: 1) anterior cingulate (ACC) regions A25, A32, A33 and A24; 2) mid-cingulate (MCC) region A24; and 3) the retrosplenial (RSC) regions A29 and A30. Of particular note, both the infralimbic and prelimbic prefrontal cortices have been assigned to anterior cingulate regions A25 and A32V respectively. The cingulum bundle is a distinct white matter tract that projects along the dorsal surface of the corpus callosum. This prominent white matter tract interconnects cortical sites as well as connecting subcortical nuclei to the cingulate cortices (Bubb, Metzler-Baddeley, & Aggleton, 2018).

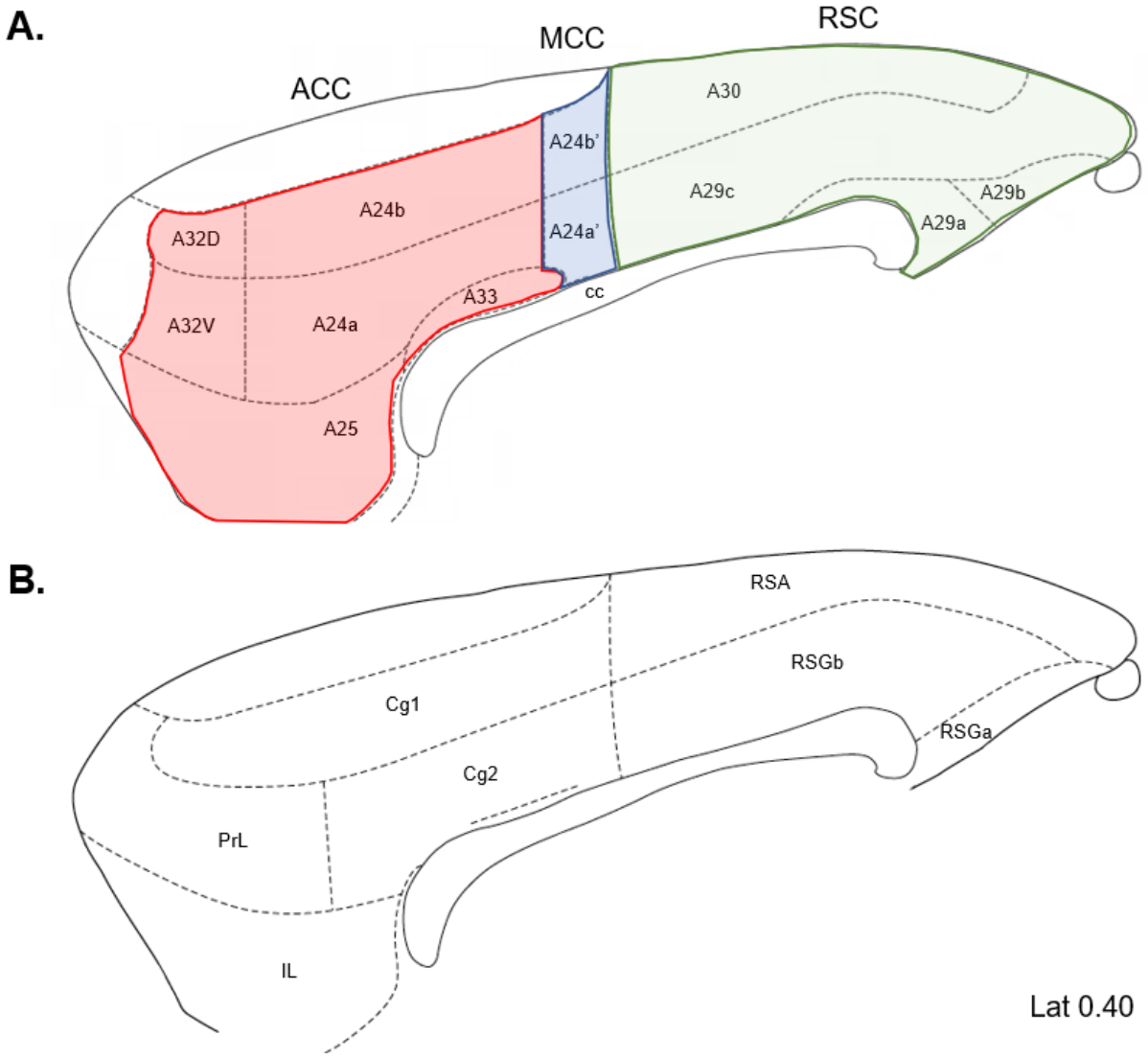

Figure 3.4. Comparison of the sagittal anterior, mid- and posterior cingulate regions. **A**. Section plate modified from Paxinos and Watson's The Rat Brain in Stereotaxic Coordinates, 7th Edition (Paxinos & Watson, 2014) and **B**. Section plate from Paxinos and Watson's The Rat Brain in Stereotaxic Coordinates, 4th Edition (Paxinos & Watson, 1998). A25 = anterior cingulate area 25 ;A32D/A32V= dorsal and ventral anterior cingulate area 32; A33 = anterior cingulate area 33; A24b/A24a = anterior and mid cingulate cortex area 24; A30 = dysgranular retrosplenial cortex area 30; A29a/b/c = granular a/b/c regions of the retrosplenial cortex area 29; ACC = anterior cingulate cortex; cc = corpus callosum; Cg1/2 = cingulate areas 1 and 2; PrL= prelimbic cortex; IL = infralimbic cortex; MCC = mid-cingulate cortex; RSA = dysgranular retrosplenial cortex; RSC = retrosplenial cortex; RSGa/b = granular a/b areas of the retrosplenial cortex. Atlas number (in mm), relative to midline, denotes the laterality of both sections.

Parahippocampal region

The parahippocampal region consists of the caudal cortical regions surrounding the hippocampus including areas of the rhinal sulcus in the rat (Burwell, 2000).Together with the HF, they comprise the "medial temporal lobe" (Squire & Wixted, 2011). This region encompasses the presubiculum, the parasubiculum, the entorhinal cortex, the perirhinal cortex and the postrhinal cortex. The perirhinal cortex is the region directly surrounding the rhinal sulcus, shown by the dotted line in Figure 3.5. Both the entorhinal and postrhinal cortices extend further posterior of the perirhinal cortex. The subicular parahippocampal regions are adjacent to the hippocampus.

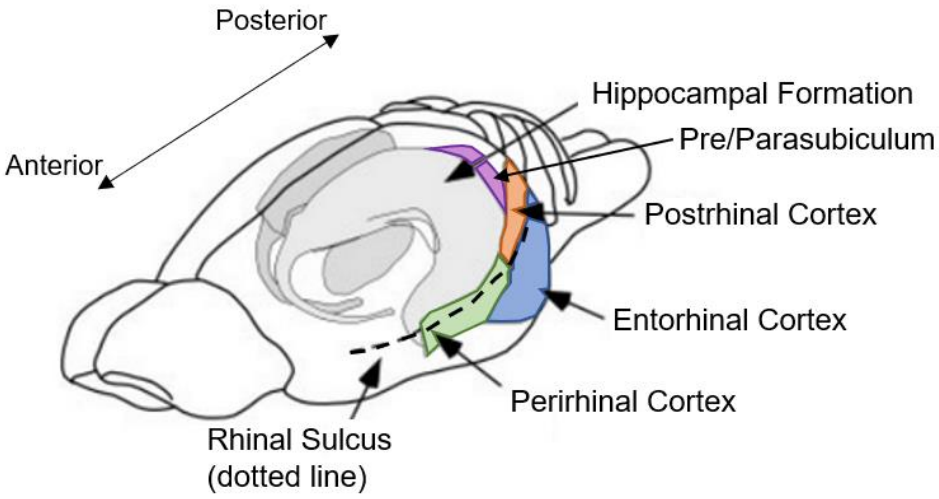

Figure 3.5. Parahippocampal region of the rat brain. Image adapted from Furtak, Wei, Agster, and Burwell (2007)

3.3 Connectivity within the 'hippocampal-diencephalic-cingulate' network

Direct efferent projections from the hippocampus to the MB arise from the subiculum, presubiculum and postsubiculum. These project mostly via the fornix before descending the PCFx (Allen & Hopkins, 1988; Wright, Erichsen, Vann, O'Mara, & Aggleton, 2010). Notably, no direct return projections are evident from the MB back to hippocampal structures. The efferent projections of the descending limb of the PCFx are topographically

organised throughout the medial and lateral mammillary nuclei. Efferent projections to the medial mammillary nuclei originate in the mid-cell layer across the proximal-distal plane of the subiculum (Christiansen et al., 2016), while lateral mammillary nuclei projections originate in the presubiculum and postsubiculum (van Groen & Wyss, 1990a). Dorsal-ventral organised subicular efferent projections are also evident. For example dorsal subicular projections terminate in the dorsal parts, and ventral subiculum projections terminate in the ventral parts of the medial mammillary nucleus (Hopkins, 2005).

As stated above, the MB distribute unidirectional projections to the ATN via the MTT. These projections consist of: 1) ipsilateral efferents from the medial mammillary nucleus to both the AV and AM, and 2) both ipsilateral and contralateral efferents from the lateral mammillary nucleus to AD (Bubb et al., 2017). Projections to the ATN from the MB primarily follow a horizontal pattern with medial mammillary projections terminating in the AM and lateral projections terminating in the AV (Shibata, 1992). As a consequence, inputs from both the dorsal and ventral subiculum to the MB probably also directly influence the parallel areas in the anterior thalamus.

Unlike the Papez (1937) depiction of the limbic circuitry, there are also dense direct projects to the ATN from subicular structures via the remaining parts of the PCFx and less densely via the internal capsule seen in **C**, Figure 3.2 (van Groen & Wyss, 1990a). A tracing study by Dillingham and colleagues (2015) showed that there are weak, nonfornical projections via the internal capsule from the distal subiculum and presubiculum that terminate in the dorsolateral AV. Dense projections to the AM and AV from the subiculum predominantly follow the PCFx (Dillingham, Erichsen, et al., 2015).

In summary, current evidence shows that there are bidirectional connections, which Papez originally considered to be unidirectional (**A**, Figure 3.2), between the ATN and the cingulate cortices and the ATN and hippocampal regions. ATN efferents are almost exclusively ipsilateral, with only a small fraction of AV cells projecting contralaterally to the RSC, at least in the rat (Mathiasen, Dillingham, Kinnavane, Powell, & Aggleton, 2017). Conversely, projections originating from the RSC and subiculum appear to terminate in both ipsilateral and contralateral ATN (Mathiasen et al., 2017). The ACC (A24) receives intermediate strength projections exclusively from restricted parts of the AM; returning projections from the prelimbic (A32V) and anterior cingulate cortices terminate in the AV and AM (Mathiasen et al., 2017; Shibata & Naito, 2005).

Of the regions within the cingulate cortex, the RSC has the strongest connections with the ATN (Shibata, 1993) through dense cingulum and internal capsule interconnections (**C**, Figure 3.2). All anterior thalamic nuclei project to the RSC in a topographically organised pattern (Shibata, 1993; Shibata & Kato, 1993; van Groen & Wyss, 1990b, 1992, 2003). Granular regions (A29a-c) of the RSC are reciprocally connected with the AV, whereas the AD projects to and receives light return projections from A29c granular cortex (van Groen & Wyss, 1990b, 2003). Projections from the AV primarily terminate in the superficial Layer I of A29c while AD projections terminate in the deeper (Layers II/III) layers as well as Layer I. AM projections, however predominantly terminate in Layers I and V of A30 (Shibata, 1993; van Groen & Wyss, 1992).

The cingulate cortex, again predominantly the RSC, projects back to parahippocampal regions. Both the granular and dysgranular RSC project densely to the pre- and postsubiculum and the medial and lateral entorhinal cortices via the cingulum. The anterior

cingulate, however, has much more limited projections back to the perirhinal and lateral entorhinal cortices (B. F. Jones & Witter, 2007). The presubiculum and postsubiculum complete a parallel pathway back to the HPC via dense entorhinal projections (van Groen & Wyss, 1990a). These entorhinal projections in turn, terminate in the dentate gyrus and CA3 regions of the HPC from entorhinal Layer II and the subiculum and CA1 from Layer III (Furtak et al., 2007). These cingulate projections to hippocampal and parahippocampal regions, in a sense, complete this notional circuit described by Bubb and colleagues (2017).

The extensive review of literature combined with new tract tracing evidence presented by Bubb and colleagues (2017) provides evidence that connectivity within this network is more complex than previously understood. Rather than the serially defined 'hippocampal-cortical-hippocampal' circuit initially described in Papez (1937), many parallel connections reinforce the notion of an extensive integrated system of structures critical for memory. This shift to an integrated extended memory system helps to expand the notion that sites such as the ATN and mammillary bodies may make their own contributions to learning and memory and are not solely relays within the system for functions of the hippocampus (Dillingham, Frizzati, Nelson, & Vann, 2015; Wright, Vann, Aggleton, & Nelson, 2015). This thesis, therefore, has focus on the wider network of structures implicated in learning and memory (Chapter 5 & 6). The thesis also focuses on the implication of damage to one of these diencephalic structures on the wider system, specifically the ATN (Chapter 7). The reason for the focus on ATN lesions is that many sources of evidence suggest it provides a key hub within the 'hippocampal-diencephalic-cingulate' network ad plays a critical, active role in supporting memory.

Chapter 4.
Anterior Thalamic Lesion Models of Diencephalic Amnesia

This chapter briefly describes some memory tasks that have been used to model episodic memory in animals. This includes a brief consideration of consolidation of memory, using tests of recent versus remote memory. A key focus is to review the effects of anterior thalamic nuclei (ATN) lesions in rats as a model of diencephalic amnesia. Evidence summarised in Chapter 2 shows that even localised human pathology extends beyond a single region. Animal lesion experiments provide the advantage of well described localised damage from post-mortem examination, comparisons of pre- and post-surgery performance, and planned memory tasks. One important feature of ATN lesions is their impact on the normal function of distal regions. In this respect, immediate early gene (IEG) changes reflect lesion effects on neural activation following injury. I will focus on the IEG Zif268. From this summary, there is clearly a need to examine the non-spatial tasks with respect to the effects of ATN and memory. Paired-associate memory tasks are generally regarded as better models of episodic-like memory than standard spatial tasks. The chapter therefore also outlines spatial and non-spatial paired-associate tasks that have been used in rats following lesions to the 'hippocampal-diencephalic-cingulate' network (Bubb et al., 2017). The conclusion is that new non-spatial paired-associate memory tasks are needed to assess the full impact of ATN lesions.

4.1 Episodic-like memory in animals

Episodic memory is arguably the most significant type of memory in human cognition. It encompasses integrated aspects of the "what", "where" and "when" memory. It generally represents the human ability to recollect a unique event from the person's own perspective.

For humans, this involves conscious recollection, and thus requires autonoetic memory, the self-awareness that remembered events are different from both the present and a feeling of familiarity, plus a sense of subjective time (Tulving, 2002). Such specific self-awareness is presumably unique to humans. Work by Clayton and Dickinson (1998), however, has shown that animals rely on episodic-like memory representations in terms of "what", "where" and "when", which suggests that we can evaluate similar processes in animals, without the need to evaluate self-awareness. When foraging for and storing food caches, scrub-jays were able to remember both the location of the preferred as opposed to less preferred food, as well as the time that the food had been placed in a given location. Scrub jays prefer wax worms, which spoil quickly, rather than peanuts, which stay fresh for longer. These researchers showed that these birds could cache peanuts and show different responding to cached wax worms in a different, visuospatially distinct location five days later. In a choice test four hours after hiding the wax worms, the birds returned to the wax worm and not the peanut cache. When scrub jays were allowed to store the food caches in the opposite order, they returned to the peanut cache as the wax worms placed five days earlier would be spoilt. Preference for the wax worm only when it was the most recent cache demonstrated memory of where and when a particular food item (i.e. "what") was cached. This combination or association of elements satisfied the behavioural criteria for episodic-like memory in non-humans (Clayton & Dickinson, 1998). Extensive animal research has established that species other than humans use these features of episodic memory and can remember information including where events took place, what happened, and the temporal order of these events.

Since the early 2000's there has been increasing focus on demonstrating episodic-like memory in rats. Eacott and Norman (2004) investigated memory of an object, its spatial location and the context in which it was presented. Intact rats demonstrated a clear preference

for exploring novel object configurations (location and context) over an objects in familiar configuration. This preference was apparent following delays of 15 minutes up to 1 hour. The preference for novelty, for both object and location, and the use of context as a temporal occasion, indicates a rat's ability to use episodic-like memory. Eacott and Norman (2004) proposed that in episodic memory, "when" serves only to set the occasion to distinguish experiences from one another. Defining the temporal component of episodic-like memory at least in rats as "which" (Eacott & Gaffan, 2005; Eacott & Norman, 2004). Kart-Teke and colleagues (2006), also developed a task to demonstrate that rats are able to form integrated memory for "what", "where" and "when/which". They combined three different versions of a novelty-preference paradigm into a single experience, i.e. object recognition, object-in-location, and temporal order. Rats demonstrated an ability to recognise objects previously explored and their order of presentation. Concurrently, the rats were also able to alter responding following a spatial displacement dependent on whether an "old familiar" or "recent familiar" object ("what") was shifted to a new location ("when" and "where"). Intact rats spent more time exploring the two old familiar objects, showing their ability to remember the previously encountered "what" and "when" components of the task. These rats also showed preference for spatially displaced "old familiar" objects than "old familiar" objects that had not been displaced, demonstrating memory for "what" and "where" (Binder, Dere, & Zlomuzica, 2015).

A deficit in just one component of "when", "where", and "what" may, however, be sufficient to impair behaviour on a task. In that circumstance, the integration of all three components of episodic-like memory, therefore, does not allow the researcher to determine the exact role of the region on components of episodic-like memory if a deficit arises (Aggleton, 2014). One solution to this problem is to use tasks that test specific elements of

memory, such as spatial ("where") working memory ("when") tasks. Spatial working memory tasks allow for the integration of within session "where" and "when" information to guide behaviour in the immediate future. Intact animals are likely to utilise a combination of allocentric (distal) spatial cues to solve spatial working memory tasks (Aggleton & Nelson, 2015). This does not, of course, address the longer-term aspect of episodic memory. Acquisition of allocentric spatial memory, including spatial working memory, has consistently been demonstrated to be sensitive to lesions of memory-related structures, unlike egocentric acquisition strategies (Aggleton & Nelson, 2015). Studies have consistently demonstrated the significance of the hippocampus (HPC) for these simpler episodic-like memory tasks (Aggleton, 2014) with disconnection studies indicating that both the anterior thalamus and retrosplenial cortex are also required to support spatial learning (Henry, Petrides, St.-Laurent, & Sziklas, 2004; Warburton, Baird, Morgan, Muir, & Aggleton, 2001). This involvement of extra-hippocampal regions now supports the view of a 'hippocampal-diencephalic-cingulate' system that supports spatial learning and memory, and episodic memory more generally (Bubb et al., 2017).

In rats, the most common examples of spatial working memory tasks are a variety of tasks in the radial arm maze (RAM), delayed non-matching to place in the T-maze, and delayed matching to place in the water maze (Morris, 1983; Olton, Becker, & Handelmann, 1979; Rawlins, 1985). These tasks all require intact allocentric spatial memory, in particular. They provide examples of "when" and "where" memory that engage neural structures presumed to support episodic-like memory. The T-maze non-matching to place task, consisting of a sample phase and a test phase, relies on the rat's tendency to alternate spatial responses for food. During the sample phase, the rat starts from a central stem with one of the two arms obstructed, directing the rat down the remaining unobstructed arm for the reward.

After a delay, the rat restarts from the central stem but is now allowed a free choice between the two arms and rewarded only in the previously blocked arm (i.e. non-matching to sample procedure; Aggleton and Nelson (2015). The water maze uses a large circular pool with a hidden platform below the surface of the opaque water. In this task, naturally, the rat is motivated to escape by locating the platform. The spatial working memory water maze procedure (matching-to-sample) often allows the rat to experience has two or more trials per day with the platform in the same location but, unlike the classic reference memory task, the platform location changes on a daily basis. Intact spatial working memory is measured by increasingly accurate navigation (shorter, more direct path) to the platform location after the first trial of the day. The RAM is most commonly used for spatial working memory and consists of a central hub with generally 8 or 12 arms extending from it. The use of guillotine doors to control movement between the central hub and the arms encourages spatial strategies, as opposed to choosing on the basis of orientation when no arms are used. In the standard RAM task, each arm is baited only once and the rat is required to visit all arms without revisits, to show good within-trial recall of where it had previously visited. Each of these tasks requires intact spatial working memory but may also be influenced by non-spatial factors and can be manipulated in ways to increase the difficulty and load on working memory. These tasks have been used extensively following lesions to the extended memory system (Aggleton, 2008; Aggleton & Nelson, 2015). These tasks are readily learned by intact rats that typically use external cues to generate allocentric spatial representations of their environment.

While evidence exists for a hippocampal role in working memory, as a sub-class of episodic memory, the classic literature on the hippocampal system focuses on long-term episodic memory (Ranganath, 2010). Here, the focus is long-term retention, and

consolidation, of episodic-like memory. Memory consolidation is the transformation or progression of information from an unstable state into enduring long-term memories (Dudai, 2004; Squire, 1992). The systems responsible for long-term consolidation and working memory are believed to be different, albeit that some structures like the hippocampus will be involved in both. Consolidation requires reorganisation at both synaptic and systems levels (Frankland & Bontempi, 2005). Synaptic consolidation occurs within hours of acquisition, involving the strengthening of new synaptic connections and reorganisation of existing ones. Synaptic activation starts the recruitment of second messenger systems (intracellular signalling molecules), activation of transcription factors and finally, the synthesis of proteins required for the structural alterations (Ledoux, 2002; Squire & Kandel, 2003). Manipulations affecting any part of this process, whether behavioural, pharmacological or genetic, blocks initial memory acquisition. Systems consolidation, by contrast, refers to a slower, more gradual process of reorganization within the memory structures in the brain (Dudai, 2004; Squire & Kandel, 2003). There are two key models of systems consolidation, the standard consolidation theory and the multiple trace theory. The standard consolidation theory suggests that there is a time dependent reorganisation of the systems supporting memory recall (Figure 4.1). The generally-accepted viewpoint is that, over time, the role of the hippocampus weakens, leaving extra-hippocampal/cortical regions to develop new or stronger connections, which then become independently capable of storing and retrieving memory traces (Frankland & Bontempi, 2005; McClelland, McNaughton, & O'Reilly, 1995; Squire & Kandel, 2003). This model is based on the assumption that episodic memories, a subset of “declarative memory”, eventually become independent of the HPC. Behavioural models of systems consolidation through the testing of recall at different time points, combined with measures of neural activation may reveal time-dependent shifts in recruitment of memory related structures in a range episodic-like tasks.

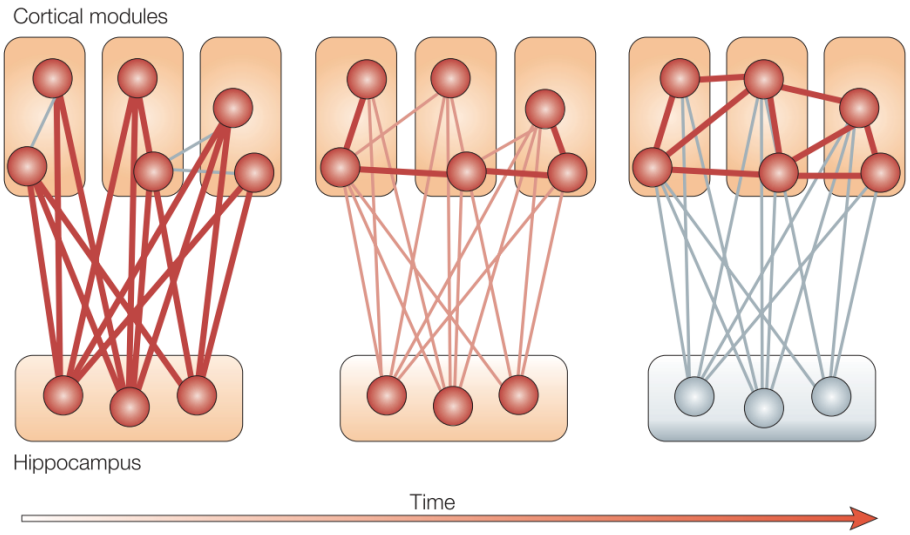

Figure 4.1. The time-dependent reorganisation of systems supporting memory recall from HPC-cortical to cortical-cortical (Frankland & Bontempi, 2005). Initial encoding of information (left) occurs in cortical areas while the hippocampus integrates information from the distributed cortical modules. Reactivation (i.e. continued training of a task) of this hippocampal–cortical network leads to progressive strengthening of cortico-cortical connections (moving right across 'time')

The multiple trace theory (Nadel & Moscovitch, 1997) proposed as an alternative to the standard consolidation model, by suggesting that the HPC is essential for contextual and spatial episodic memory, irrespective of time (Figure 4.2). Although memory traces are initially encoded in a similar manner to the standard model but, following reactivation or re-exposure, traces remaining in the HPC provide spatial and temporal context (episodic-like memory) while traces in the cortex are context free (semantic). This multiple trace theory argues that both spatial and temporal information that constitute the memory depend on the continued involvement of the hippocampus and the prefrontal cortex. This would therefore suggest that complete HPC lesions or disconnection of the HPC-PFC pathways will continue to produce a degree of retrograde amnesia. While there is clinical and animal evidence for both models, there is on-going debate as to how the time-dependent transfer of memories

occurs. Both the standard and multiple trace models agree that there are time-dependent shifts in the organisation of memory traces. However, there is still debate as to whether episodic, context-rich memories always become independent of the hippocampus over time. Further investigation into episodic-like memory retrieval is required to determine neural activation for context rich memory.

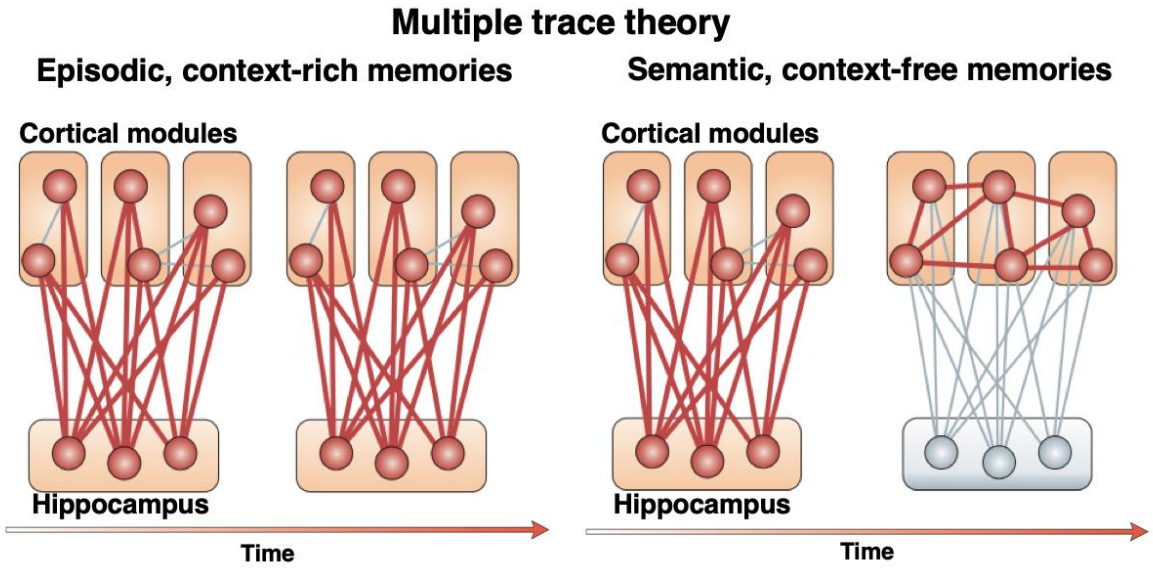

Figure 4.2. Multiple trace model of memory consolidation (Adapted from Frankland and Bontempi, 2005). Both episodic and semantic memory require cortical and hippocampal involvement. The main difference of this theory is that over time hippocampal activation is reduced for semantic memory only.

4.2 Anterior Thalamic Lesion Studies

Lesions to the rat ATN produce robust memory deficits that presumably reflect their influence as an key node with diverse neural connections in a hippocampal-diencephalic-cingulate network (Bubb et al., 2017). Table 4.1 summarises the effects of 38 ATN lesion studies. It is clear that there has been a focus on testing spatial memory in this research.

Table 4.1. Summary of ATN lesion behavioural studies.

Author (Date)	Lesion Site	Method	Species	Behavioural Tasks	Deficits
Aggleton et al. (1996)	MD; AV/AD; AM; ATN	NMDA	Rat (DA Strain)	T-maze forced alternation	AM and AV/AD slow acquisition; ATN impaired
				Allocentric alternation	ATN impaired
				Egocentric discrimination	Not impaired
				8-arm RAM (90° rotation)	AV/AD and ATN impaired
Byatt and Dalrymple-Alford (1996)	AV; AM	RF	Rat (Wistar)	12-arm RAM	AV and AM impaired
				12-arm RAM no intra- or extra-maze cues	AV and AM impaired
Sziklas and Petrides (1999)	ATN	Electrolytic	Rat (Hooded)	8-arm RAM	Impaired
				Object-place association	Impaired
				Visuo-spatial (egocentric)	Not impaired
Warburton and Aggleton (1999)	ATN; FX	NMDA; RF	Rat (DA Strain)	Water maze	ATN and FX impaired (ATN worse)
				T-maze forced alternation	ATN and FX impaired (ATN worse)
				Spontaneous object recognition	Neither impaired
Warburton, Morgan, Baird, Muir, and Aggleton (1999)	ATN; ATN+(MD, ventral and midline nuclei); FX	NMDA; RF	Rat (DA Strain)	Morris water maze	ATN and FX impaired, but reacquired; ATN+ permanently impaired
				T-maze forced alternation	ATN+ impaired
Warburton, Baird, Morgan, Muir, and Aggleton (2000)	ATN+FX (ipsi); ATN+FX (contra); ATN+FX (contra) + HPC	NMDA	Rat (DA Strain)	Spontaneous object recognition	No impairment
				Object-in-place	All impaired
				T-maze forced alternation	
				Water maze	
				8-arm RAM	
				T-maze alternation	
Chudasama, Bussey, and Muir (2001)	PrL; MD; ATN	NMDA	Rat (Lister Hooded)	Visual discrimination and reversal	MD impaired, PrL & ATN not impaired
Alexinsky (2001)	ATN; MD; RSC; PPC	Ibotenic, excision	Rat (Sprague-Dawley)	3/8 baited RAM (reference and working memory)	MD less correct visits, ATN more incorrect reference/working memory visits
				New route (pre-exposure – Y/N)	ATN, MD, PPC impaired
				Contextual light change	ATN most repetitive errors
Ward-Robinson et al. (2002)	ATN	NMDA	Rat (DA Strain)	Non-spatial sensory preconditioning to fear	Not impaired
				Conditioned taste aversion	Not impaired
				T-maze forced alternation	Impaired

Author (Date)	Lesion Site	Method	Species	Behavioural Tasks	Deficits
van Groen, Kadish, and Wyss (2002)	AD/AV; AD/AV+; ATN	Ibotenic	Rat (Sprague-Dawley)	Water maze	All impaired – ATN no learning across trials
Mitchell, Dalrymple-Alford, and Christie (2002)	ATN	Scopolamine	Rat (Female - Hooded)	12-arm RAM (infused after 6 forced visits – infusions of 1, 2.51, 6.31, 10, 15µg)	10µg increased errors in both forced and free choice/working memory errors.
R. G. Mair, Burk, and Porter (2003)	ATN; PH	NMDA; RF	Rat (Long-Evans)	Delayed non-matching to sample RAM	ATN and PH impaired
Moran and Dalrymple-Alford (2003)	ATN; PRh	NMDA	Rat (Female - Hooded)	12-arm RAM	ATN impaired
				Spatial configuration	PRC impaired
				Spontaneous object recognition	Neither impaired
Corbit, Muir, and Balleine (2003)	MD; ATN	NMDA	Rat (Long-Evans)	Instrumental conditioning	Neither impaired
				Devaluation extinction	MD impaired
Henry et al. (2004)	ATN(unilat)+HPC	Electrolytic	Rat (Hooded)	Spatial conditional associative task	Impaired
				Delayed forced alternation	
Sziklas and Petrides (2004)	ATN; HPC; MB	Electrolytic	Rat (Hooded)	Egocentric conditional associative task in a T-maze	HPC impaired; ATN & MB not impaired
Mitchell and Dalrymple-Alford (2005)	ATN; LT; MT	NMDA	Rat (Hooded)	12-arm RAM	ATN impaired
				Memory for reward magnitude	MT impaired
				Temporal order memory	LT and MT impaired
				Familiar vs. novel object recognition	No impairment
Wolff et al. (2006)	ATN	NMDA	Rat (Female - Hooded)	Temporal order memory for odour	Severely impaired
Mitchell and Dalrymple-Alford (2006)	ATN; LT	NMDA	Rat (Female - Hooded)	Response working memory in a plus maze	LT impaired
				Spatial working memory in an 8-arm RAM	ATN impaired
Gibb, Wolff, and Dalrymple-Alford (2006)	ATN; LT; MT	NMDA	Rat (Female - Hooded)	Odour-placed paired-associate task	ATN and LT impaired; MT not impaired
				Spatial and odour discrimination	No impairment

Author (Date)	Lesion Site	Method	Species	Behavioural Tasks	Deficits
Sziklas and Petrides (2007)	ATN	Electrolytic	Rat (Hooded)	Visual-spatial conditional associative learning	No impairment
				8-arm RAM	Impaired
Wolff, Gibb, Cassel, and Dalrymple-Alford (2008)	ATN; ILN	NMDA	Rat (Hooded)	Allocentric spatial reference memory in water maze	ATN impaired; ILN no impairment
				Y-maze discrimination	No impairment
Wolff, Loukavenko, Will, and Dalrymple-Alford (2008)	ATN	NMDA	Rat (Hooded)	Water maze spatial reference memory, fixed release point	ATN impaired acquisition
				Water maze spatial reference memory, variable start points	
Aggleton, Poirier, Aggleton, Vann, and Pearce (2009)	ATN; Fx	NMDA; RF	Rat (DA Strain)	Configural learning	ATN and Fx not impaired
				Geometric learning	ATN impaired; Fx not impaired
				T-maze spatial alternation	ATN and Fx impaired
Lopez et al. (2009)	ATN; ILN/LT	NMDA	Rat (Long-Evans)	Morris water maze acquisition and delayed re-testing	ATN impaired on all conditions; ILN/LT impaired only on 25-day delay condition
Dumont, Petrides, and Sziklas (2010)	Fx+RSC ipsi; ATN+HPC+RSC contra; ATN+HPC+RSC ipsi	Ibotenic; Electrolytic (Fx only)	Rat (Hooded)	Visuospatial conditional associative task	All impaired
				8-arm RAM	
Aggleton et al. (2011)	ATN	NMDA	Rat (Lister Hooded)	Sequence learning	No impairment
				T-maze – spatial alternation	Impaired
Moreau et al. (2013)	ATN; ILN/LT	NMDA	Rat (Long-Evans)	Water maze – spatial learning	ATN impaired
				Water maze- visual discrimination	No impairment
Dumont and Aggleton (2013)	ATN	NMDA	Rat (Hooded)	T-maze alternation	Severe impairment
				Object recognition	No impairment
				Object recency	Impairment on within- but not between-block recency
Alcaraz et al. (2014)	ATN; MD	NMDA (pressure injected)	Rat (Long-Evans)	T-maze (goal oriented)	ATN and MD impaired
				T-maze forced alternation	ATN impaired; MD not impaired
				Operant box (progressive ratio)	ATN impaired due to poor spatial ability; MD impaired in adaptation of choice according to goal value

Author (Date)	Lesion Site	Method	Species	Behavioural Tasks	Deficits
Dumont, Amin, and Aggleton (2014)	ATN	NMDA	Rat (Hooded)	Biconditional discriminations (nose pokes)	No impairment
				Biconditional learning (open arena digging)	No impairment in learning the biconditional task, severely impaired in acquiring association (specific cup and context).
				Spatial go/no-go discrimination	Impaired
Harland, Collings, McNaughton, Abraham, and Dalrymple-Alford (2014)	ATN	NMDA	Rat (Hooded)	T-maze	Severe impairment - ameliorated with ENR
				RAM	Severe impairment - ameliorated with ENR
Ulrich, Aitken, Abraham, Dalrymple-Alford, and McNaughton (2014)	ATN	NMDA	Rat (Long-Evans)	T-maze (with short and long breaks)	Impairment in reacquisition post-surgery in both short and long break
Loukavenko et al. (2015)	ATN	NMDA	Rat (Female - Hooded)	T-maze (saline, ENR, and cerebrolysin tx)	Saline tx - severe impairment; ENR reduced deficit; cerebrolysin reduced deficit; combined ENR + cerebrolysin greater improvement even with addition of 40 sec delay
Wright et al. (2015)	ATN	NMDA	Rat (Lister Hooded)	Intra-dimensional attentional shifting task	ATN significantly impaired in acquisition of the task
				Extradimensional attentional shifting task	ATN impaired - required more trials to overcome extra-dimensional shift
Perry et al. (2018)	ATN; MTT	NMDA; RF	Rat (Hooded)	Water maze spatial reference memory	ATN impaired; MTT not impaired
				Water maze spatial working memory (repeated with reduced spatial cues)	ATN and MTT impaired
				8-arm RAM	ATN severely impaired; MTT mildly impaired
				8-arm RAM (60s mid-trial delay)	ATN improved slightly with delay; MTT greater impairment with delay
				8-arm RAM (45^{o} rotation)	ATN severely impaired; MTT impaired

Author (Date)	Lesion Site	Method	Species	Behavioural Tasks	Deficits
Kinnavane, Amin, Aggleton, and Nelson (2019)	ATN	NMDA	Rat (Lister Hooded)	T-maze alternation	ATN impaired
				Strategy shift (operant box)	
				Visual discrimination	Not impaired
				Switch to response	Impairments only in first sessions
				Response reversal	Not impaired
				Visual reversal	
				Strategy shift (water tank)	
				Response discrimination	Not impaired
				Response reversal	Impaired
				Switch to visual	Not impaired
				Visual reversal	
				Response conflict (Stroop; operant box)	
				Conditional discrimination	Not impaired
				Congruent trials (no conflict)	
				Incongruent trials (conflict)	Impaired in first 10s of presentation
Frost et al. (2020)	ATN	NMDA, muscimol	Rat (Lister Hooded)	Spatial alternation	ATN impaired
				Bow tie maze object recognition	Not impaired

Abbreviations: ATN = anterior thalamic nuclei; AD = anterodorsal thalamic nuclei; AM = anteromedial thalamic nuclei; AV = anteroventral thalamic nuclei; CNQX: cyanquixaline; contra = contralateral; DA Strain = Dark Agouti rat strain; ENR = enrichment; Fx = fornix; HPC = hippocampus; ILN = intralaminar nuclei; ipsi = ipsilateral; LT = lateral thalamic aggregate; MB = mammillary bodies; MD = mediodorsal thalamic nuclei; MT = posteromedial thalamic aggregate; NMDA = N-methyl-D-Aspartate; PH = parahippocampal cortex; PrL = prelimbic cortex; PPC = posterior parietal cortex; PRh = perirhinal cortex; RF = radiofrequency; RSC = retrosplenial cortex; tx = treatment; unilat = unilateral

Table 4.1 demonstrates the well-established role of the ATN in spatial memory tasks. All but two of the 38 studies in Table 4.1 examined allocentric spatial learning tasks, all finding deficits after ATN lesions. One study investigated egocentric (intra-maze and body turn information) spatial learning only (Sziklas & Petrides, 2004). Deficits in this study were confined to lesions of the HPC, whereas rats with either ATN or MB lesions did not differ from controls. The remaining study compared ATN, PrL and MD lesions on a visual discrimination and reversal learning tasks using computer generated images in an operant chamber (Chudasama et al., 2001). Only MD lesion deficits were found on acquisition learning and reversal. None of the 10 studies that investigated resulted in lesion effects on non-spatial tasks, despite spatial working memory being impaired. These non-spatial tasks included object recognition, configural (biconditional) learning and visual discrimination (Aggleton et al., 2009; Dumont & Aggleton, 2013; Dumont et al., 2014; Kinnavane et al., 2019; Mitchell & Dalrymple-Alford, 2005; Moran & Dalrymple-Alford, 2003; Moreau et al., 2013; Warburton & Aggleton, 1999; Warburton et al., 2000).

The most severe behavioural deficits in the T-maze are seen when egocentric cues are minimised. This can be achieved by preventing the rat from using body turn (egocentric) information in the T-maze, by embedding the 'T' within a cross maze and alternating start positions from either end of the maze between the sample and test runs for some trials (Aggleton et al., 2011; Loukavenko et al., 2015). In contrast, when rats are able to use egocentric cues in the T-maze, no behavioural deficits are seen following both full and partial ATN lesions (Aggleton et al., 1996; Sziklas & Petrides, 1999, 2004; Wolff, Gibb, et al., 2008).

Manipulations to the standard RAM task, such as the rotation of the maze during a mid-trial delay may increase the working memory load and control for the use of local cues, and

generally generate more severe deficits in the RAM. A mid-trial 45˚ rotation of the maze with rewards remaining in their relative position in the environment has been shown to produce severe impairments following complete ATN lesions (Perry et al., 2018). Aggleton et al. (1996) also provide evidence of impaired performance following a 90˚ mid-trial maze rotation, with ATN lesion groups performing worse than controls.

One class of learning and memory, namely paired-associate learning, is regarded as particularly relevant "episodic-like" memory tasks. This is because their solution requires the integration of different arbitrary elements as a representation so that the component elements can be used in a flexible manner to guide behaviour. The acquisition of such arbitrary associations is believed to be a key function of the hippocampus in both animals and humans (Aggleton & Pearce, 2001; Cohen & Eichenbaum, 1993; Eacott & Norman, 2004; Gilbert & Kesner, 2002; Ranganath, 2010; Schacter & Addis, 2009). Tasks in rats employed the integration of items (i.e. objects or odours) with place or context information to guide responding. ATN lesions have been implicated in paired-associate tasks requiring integration of stimuli within a spatial context. Gibb et al. (2006), for example, demonstrated that ATN lesions severely impaired the ability to learn the odour-place association, while performance on non-associative odour discrimination was not impaired. In this task, rats were trained to respond to correct odour-place pairings and ignore incorrect pairings on a cheeseboard apparatus. Rats with ATN lesions were unable to acquire this odour-place task even after extensive training across multiple sessions. Sziklas and Petrides (1999) provided evidence that damage to the ATN impaired performance on an object-place association task, whereas performance on an egocentric task requiring associations between an object and a body turn was spared.

One problem for this literature is that there are very few studies providing evidence of ATN involvement extending beyond spatial memory. Deficits in temporal context memory are a common neuropsychological outcome in patients with Korsakoff's syndrome (Kopelman, 2015). This indicates that recency and temporal context tasks may be particularly sensitive to lesions of the ATN. The HPC is implicated in the temporal separation of sensory events, with lesions producing memory deficits for odour sequence (Fortin, Agster, & Eichenbaum, 2002; Kesner, Gilbert, & Barua, 2002). One study by Wolff et al. (2006) examined how the ATN may compare to the previous evidence of HPC involvement in temporal order memory of non-spatial items. They found that ATN lesioned rats were unable to identify the odour that occurred earlier in the sequence of six sample trials from two simultaneously presented odours. This important study provided the first explicit evidence of a behavioural deficit following ATN lesions on a hippocampal-dependent task that was independent of any spatial memory requirement. These temporal order, or relative recency, effects have been extended to object tasks, which suggest that the effect concerns the separation of closely related items in time (i.e. within-block), not between separate episodes (i.e. between block) (Dumont & Aggleton, 2013).

Despite extensive evidence that ATN lesions produce deficits in many tasks, Wright et al. (2015) provided evidence to show that ATN lesions may facilitate performance in an attentional set-shifting task. The involvement of the ATN was examined in attentional processes typically supported by the PFC. ATN lesions reduced acquisition for discriminations based on relevant stimulus (intradimensional) but facilitated performance on irrelevant stimulus (extradimensional shift) trials. Wright et al (2015) suggested that these results indicate that the ATN have a role in guiding non-spatial attention. It was suggested that spatial strategies used by intact rats may impede performance on this task while ATN lesioned rats are not burdened by these strategies.

Kinnavane et al. (2019) also investigated the impact of ATN lesions on behavioural flexibility. Such tasks that are typically associated with the PFC and ACC function. Rats with ATN lesions were tested in both an operant box and water tank on their ability to update behavioural responding as reward contingencies were changed. Rats with ATN lesions performed similarly to controls for visual discrimination and reversal tasks in an operant box and water maze. ATN lesions did appear to slow acquisition for response discriminations, impair the reversal of spatial discrimination and reduce responding on a response conflict (Stroop) task, concluding that these non-spatial deficits in ATN lesion rats are aligned to evidence of ACC damage.

In summary, evidence across numerous studies reveals that memory impairments following ATN damage have focused on spatial memory tasks requiring the use of external, or allocentric, spatial representations. Unilateral crossed lesions between the ATN and the hippocampus and fornix support the idea that these systems have a degree of inter-dependency (Henry et al., 2004; Warburton et al., 2000). The strongest evidence of ATN involvement beyond the spatial domain concerns the relative recency (temporal discrimination) of items such as odours and objects (Dumont & Aggleton, 2013; Wolff et al., 2006). These deficits support the interdependence of structures within the 'hippocampal-diencephalic-cingulate' memory system (Bubb et al., 2017), specifically the ATN and hippocampus. Paired associate learning, discussed below, is another option to study the involvement of the ATN in complex memory tasks. Other than a few instances, the literature on paired-associate learning has generally focused on lesions outside the ATN. So, we will first address the neural impact of ATN lesions on other neural structures in the extended memory system that was described in Chapter 3.

4.3 Neural activation and distal changes following ATN injury

A noteworthy feature of ATN lesions is that they reliably produce "covert pathology" in distal regions of the 'hippocampal-diencephalic-cingulate' network (Bubb et al., 2017). Covert

pathology refers to a structure that seems to be normal on standard histological assessment yet indicates evidence of a functional lesion (Aggleton, 2008). Immediate early genes (IEGs) constitute a group of transcription factors that are encoded rapidly following cell activation (Davis, Bozon, & Laroche, 2003). They are therefore suitable molecular markers of neuronal activity to reveal which neural regions are recruited after behavioural stimulation (Kubik, Miyashita, & Guzowski, 2007). A range of IEGs appear to be necessary for the consolidation of long-term memories; as memory recall is disrupted following inactivation (Davis et al., 2003).

Studies on ATN lesions, and related mammillothalamic tract lesions, have focused on two IEGs Table 4.2. The early growth response gene 1 (*Egr-1*), more commonly known as zinc finger binding protein (Zif268), is an IEG that facilitates synaptic plasticity, spatial memory and consolidation (Alberini, 2009). c-Fos protein, a proto-oncogene, is an IEG that induces cellular responses (membrane depolarisation and voltage-gated calcium influx). Like Zif268, c-Fos has been shown to facilitate spatial memory and consolidation (Méndez-Couz, Conejo, Vallejo, & Arias, 2014). In addition, the regulatory transcription factor cAMP response element binding protein (CREB) and the 'active' phosphorylated cAMP response element (pCREB) are also indicators of neural activation. pCREB is considered of particular interest due to its role in neural plasticity and involvement in regulation of IEG expression (Alberini, 2009). CREB and pCREB have been investigated after ATN lesions (Dumont ATN). An additional marker is cytochrome-oxidase (CO), which provides a more stable measure of neural metabolism associated with oxidative phosphorylation (Wong-Riley, 1989). CO has also been investigated after ATN lesions (Mendez-Lopez, Arias, Bontempi, & Wolff, 2013).

Zif268 upregulation has been attributed to various stimuli, most notable of these being memory, neurodegeneration, brain injury, apoptosis, and stress (Davis et al., 2003; Knapska & Kaczmarek, 2004). Zif268 is rapidly upregulated in response to learning and memory and

appears to be related to maintenance rather than the induction of synaptic plasticity (M.W. Jones et al., 2001). Evidence of upregulation has been reported in rats following spatial and associative learning (e.g. fear-conditioning), and association (visual) in monkeys (Guzowski, Setlow, Wagner, & McGaugh, 2001; Hall, Thomas, & Everitt, 2001; Tischmeyer & Grimm, 1999). Genetic knockout studies in mice have also shown that absence of IEG processes can produce deficits in long-term memory consolidation. Zif268 knockout mice were impaired in a range of tasks in long-term memory retention but retained short term memory integrity. These deficits have been shown in object recognition, spatial learning, and conditioned taste aversion tasks (M. W. Jones, French, Bliss, & Rosenblum, 1999).

To reduce floor effects, activation levels of IEGs are generally increased through introducing animals to novel stimuli prior to perfusion. Novel cages, testing rooms and/or procedures or new holding rooms are commonly introduced 90 minutes prior to perfusion to drive IEG expression (Dumont et al., 2012; Jenkins, Amin, Pearce, Brown, & Aggleton, 2004; Jenkins, Dias, Amin, & Aggleton, 2002; Jenkins, Dias, Amin, Brown, & Aggleton, 2002; Poirier & Aggleton, 2009). Placing animals in a dark room post-final testing procedures reduces exposure to other stimuli that may evoke task-unrelated IEG expression (Jenkins, Dias, Amin, Brown, et al., 2002).

Table 4.2 summarises distal changes using IEG and metabolic activation markers following lesions to both the ATN and MTT. Lesions to the MTT are also of interest (and included in the table) as they indirectly disconnect the RSC because they project on to the ATN yet produce broadly similar covert pathology like ATN lesions.

Table 4.2. Summary of studies examining biomarkers of neural activation following ATN and MTT lesions.

Author (Year)	Neural marker	Lesion type	Neural activity induction procedure	Regions analysed	Outcome relative to neural activity marker
Perry et al. (2018)	Zif268	ATN; MTT	90 minutes delay after final testing session in RAM	RSC, HPC, ACC, Sub	ATN (to a greater extent) and MTT lesions reduced Zif268 in superficial granular RSC and CA1. Only ATN lesions decreased Zif268 in deep granular and superficial dysgranular RSC, and the ACC
Frizzati et al. (2016)	Zif268 and CO	MTT	Forced runs in a RAM with novel cues.	CA1, CA3, DG and RSC	MTT lesions reduced Zif268 in superficial and deep RSC (granular and dysgranular) and reduced CO in the superficial dysgranular and the deep granular RSC.
Loukavenko et al. (2015)	c-Fos	ATN	90 minute delay after T-maze (after 3 trials)	PrL, IL, ACC, dHPC and RSC, sensory and motor cortex	ATN lesions reduced Fos counts in the superficial and deep granular RSC.
Dupire et al. (2013)	c-Fos and pCREB	ATN	60 minute delay after final plus maze task	amygdala (BLA and LA), HPC, Sub and RSC	ATN lesions reduced Fos and pCREB counts in the BLA and superficial and deep granular RSC. Fos counts were also reduced in the vSub and superficial ACC, and pCREB reduced in dCA1 and vCA1
Mendez-Lopez et al. (2013)	CO	ATN	90 minutes after final RAM session	PrL, IL, ACC, CA1, CA3, DG, Sub, RSC, PC, EC, CPu	ATN lesions reduced CO activity in the superficial granular RSC and superficial Cg1

Author (Year)	Neural marker	Lesion type	Neural activity induction procedure	Regions analysed	Outcome relative to neural activity marker
Vann (2013)	c-Fos	MTT PFx VTg	Forced runs in RAM with novel cues.	RSC, HPC, IL, PrL, sensory cortex, supra-mammillary nucleus, and lateral and medial septum	MTT lesions reduced c-Fos in the RSC, HPC and PL.
Dumont et al. (2012)	1. Zif268	1. Unilateral ATN	1. Novel or familiar object exploration	PrL, IL, PRC, HPC, Sub, RSC	1. Reduced Zif268 in granular RSC and post-sub. Novel object increases in HPC Zif268 not associated with ATN lesions.
	2. Zif268, CREB, pCREB and GAP-43	2. Bilateral ATN	2. Forced runs RAM with novel cues.		2. Reduced Zif268 and pCREB counts in the granular RSC and reduced Zif268 in post-sub
Poirier and Aggleton (2009)	1 and 4. c-Fos and Zif268 2 and 3. c-Fos	1. Unilateral ATN 2. Bilateral ATN 3. Unilateral ATN 4. Unilateral LD	Novel room and cage sacrificed: 1. 1, 2, 4 and 8 weeks, 2. 4 weeks or 1 year, 3. 4 weeks and 4. 3.5-4.5 months Novel room with activity cages with beams for locomotor activity	1. Rgb 2, 3 & 4. RSC	1. Highest Zif268 and c-Fos increase after 1 week, superficial RSC. Reductions in deep RSC counts at week 8 in Fos and week 4 in Zif268. 2. Reduced c-Fos counts in superficial granular at 4 weeks and superficial and deep dysgranular at 1 year. 3. Reduced c-Fos counts in granular 4. No change in c-Fos and Zif268 counts.

Author (Year)	Neural marker	Lesion type	Neural activity induction procedure	Regions analysed	Outcome relative to neural activity marker
Poirier et al. (2008)	c-Fos	Unilateral ATN	Novel room and cage with visual stimuli	Granular RSC	Reduction in c-Fos in the RSC ipsilateral to lesion side
Jenkins, Amin, et al. (2004)	1. c-Fos 2. c-Fos and Zif268	1 & 2. ATN 2. Postrhinal cortex	1. Foraging in novel room 2. Activity box in a novel room	RSC	1/2. ATN lesions reduced c-Fos in superficial RSC 2. ATN lesions reduced c-Fos and Zif268 in superficial granular and the deep RSC. No changes following postrhinal lesions
Jenkins, Dias, Amin, and Aggleton (2002)	c-Fos	Unilateral ATN	Place in dark box following spatial working memory RAM task	HPC, Sub, PH	Reduced c-Fos in dHPC, CA1, DG, post and pre-sub, and RSC ipsilateral to lesion
Jenkins, Dias, Amin, Brown, et al. (2002)	c-Fos	ATN	Spatial working memory RAM task	HPC, Sub, PL, ACC, RSC	Reduced c-Fos counts were found in the PL, ACC, RSC, HPC

Abbreviations: ACC = anterior cingulate cortex; ATN = anterior thalamic nuclei; BLA = basolateral amygdala; CA1 = area CA1 of the hippocampus; CO = cytochrome oxidase; CPu = caudate putamen; CREB = c-AMP response element binding protein; d = dorsal; DG = dentate gyrus; dHPC = dorsal hippocampus; dSub = dorsal subiculum; EC = entrorhinal cortex; GAP-43 = growth associated protein 43; HPC = hippocampus; IL= infralimbic cortex; l = lateral; LA = lateral amygdala; LD = laterodorsal thalamic nucleus; pCREB = phosphorylated c-AMP response element binding protein; PH = parahippocampal cortex; PrL= prelimbic cortex; Postrh = postrhinal cortex; post-sub = post-subiculum; PRC = perirhinal cortex; pre-sub = pre-subiculum; PC = parietal cortex; RAM = radial arm maze; RSC = retrosplenial cortex; Sub = subiculum; v = ventral; vHPC = ventral hippocampus.

Table 4.2 demonstrates the downregulation of IEG neural markers with evidence of covert pathology, most strongly and consistently in the RSC (Figure 4.2; Aggleton (2008). Bilateral ATN lesions can also cause significant decreases in cFos in the dorsal and ventral HPC as well as the mPFC (Jenkins, Dias, Amin, Brown, et al., 2002). Unilateral ATN lesions have also been shown to result in same-hemisphere hypoactivity, visualised through a reduction of Fos counts in the RSC, but also dorsal HPC, specifically in the CA1 and DG, presubiculum and postsubiculum (Jenkins, Dias, Amin, & Aggleton, 2002). While the majority of IEG effects after ATN lesions have emanated from the Cardiff laboratory, other labs have replicated the main effects. For example, after spatial memory testing, Loukavenko et al. (2015) also reported decreased c-Fos counts in the superficial and deep layers of the RSC following bilateral ATN lesions; these deficits were not corrected by postoperative housing in enriched environments, despite some improvements in spatial working memory. Not all studies report changes in the HPC, however, this may be due to different task procedures prior to sacrifice (Dumont et al., 2012; Dupire et al., 2013; Loukavenko et al., 2015). Bilateral MTT lesions have also been shown to result in hypoactivation of Fos in the RSC, as well as DG, CA1 and CA3 regions of the HPC (Perry et al., 2018; Vann, 2013).

The consistent reduction of IEG expression in the RSC (up to 80%) reflects the major direct connections with the ATN (Aggleton, 2008). Looking at both c-Fos and Zif268, Poirier and Aggleton (2009) suggested that shorter intervals between ATN lesion surgery and perfusion restricted IEG changes to the superficial layers of the RSC. With longer intervals, IEG disruption appeared to expand into the deeper layers of the RSC (Figure 4.2), indicating more global disruption with longer survival post-injury (Jenkins, Vann, Amin, & Aggleton, 2004; Poirier & Aggleton, 2009).

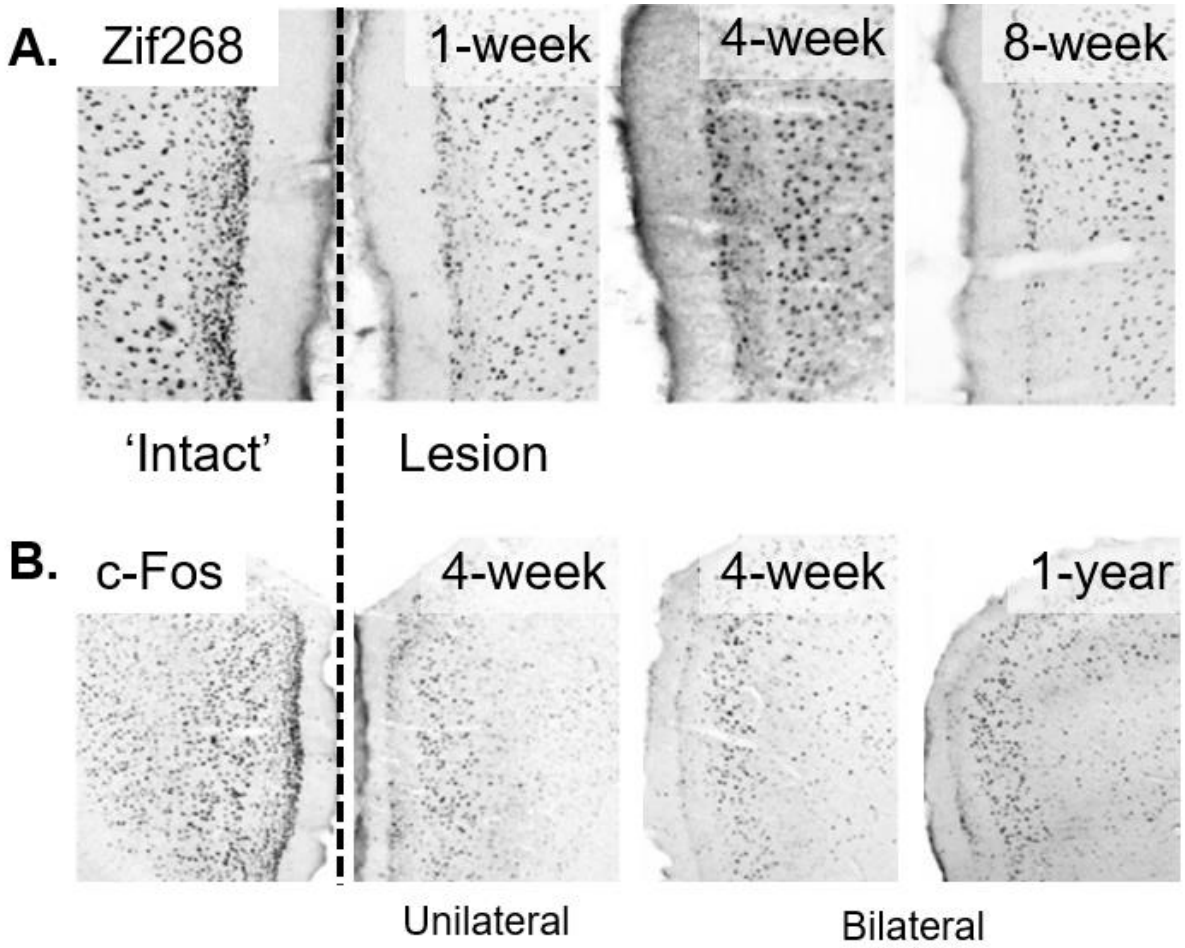

Figure 4.3. Examples of distal retrosplenial immediate early gene (**A**. Zif268 and **B**. c-Fos) changes following ATN pathology. Weeks indicate the number of weeks since introduction of ATN lesions. All Zif268 stained lesion sections show ipsilateral distal RSC effects following unilateral lesions. Adapted from (Poirier & Aggleton, 2009).

With respect to other markers, Dumont et al., (2012) found reductions in pCREB in the RSC following ATN lesions, while Dupire et al., (2013) reported reductions in the amygdala, CA1 and RSC. The difference may reflect the different tasks used across the two studies, with potential recruitment of the hippocampal-amygdalar circuit in the conditioned fear task (Dupire et al., 2013). Harland et al., (2014) found evidence of a pronounced reduction in dendritic spine densities in CA1 and RSC neurons following ATN lesions. The significant microstructural alterations are an indication of altered synaptic plasticity, with CA1 changes being the most apparent, as one of the primary HPC outputs with no direct connectivity with the ATN (Harland et al., 2014).

Frizzati et al. (2016), compared levels of the metabolic marker cytochrome oxidase and Zif268 in the RSC and HPC following MTT lesions. Rats were run on a forced-choice RAM task 90 minutes prior to perfusion in order to drive IEG expression. Although severe spatial memory deficits were found in the T-maze, MTT lesions did not reduce Zif268 expression in the HPC, in contrast to evidence of hypoactivation of c-Fos in the HPC following ATN lesions (Loukavenko et al., 2015). Reduced levels of cytochrome oxidase were found in all regions of the RSC, but no changes were found in the HPC (Frizzati et al., 2016).

Neural changes following systems consolidation

Neural activation can also be measured to determine regional differences associated with systems-level consolidation of memory. Mapping the metabolic marker (^{14}C)2-deoxyglucose levels in mice, Bontempi, Laurent-Demir, Destrade, and Jaffard (1999) were able to show a transitory role for the HPC in memory storage but evidence of activity in cortical sites associated with remote memory recall. Mice were required to learn which locations provided food rewards in 8-arm RAM. Nine daily sessions were conducted and then memory recent (5 days post task acquisition) or remote recall (25-days) was assessed. A third group was exposed to a new context at day 25 in that food rewards were now presented in new spatial locations (A, Figure 4.3). The 25-day interval between training and retention (remote recall group) was associated with low (^{14}C)2-deoxyglucose levels in the HPC and high levels in prefrontal and anterior cingulate cortices (B, Figure 4.3). Like recall at 5 days, new learning at the "remote" time-point revealed high activation in the HPC, indicating that it is a critical region for encoding of new information and the recent recall of spatial information (Bontempi et al., 1999).

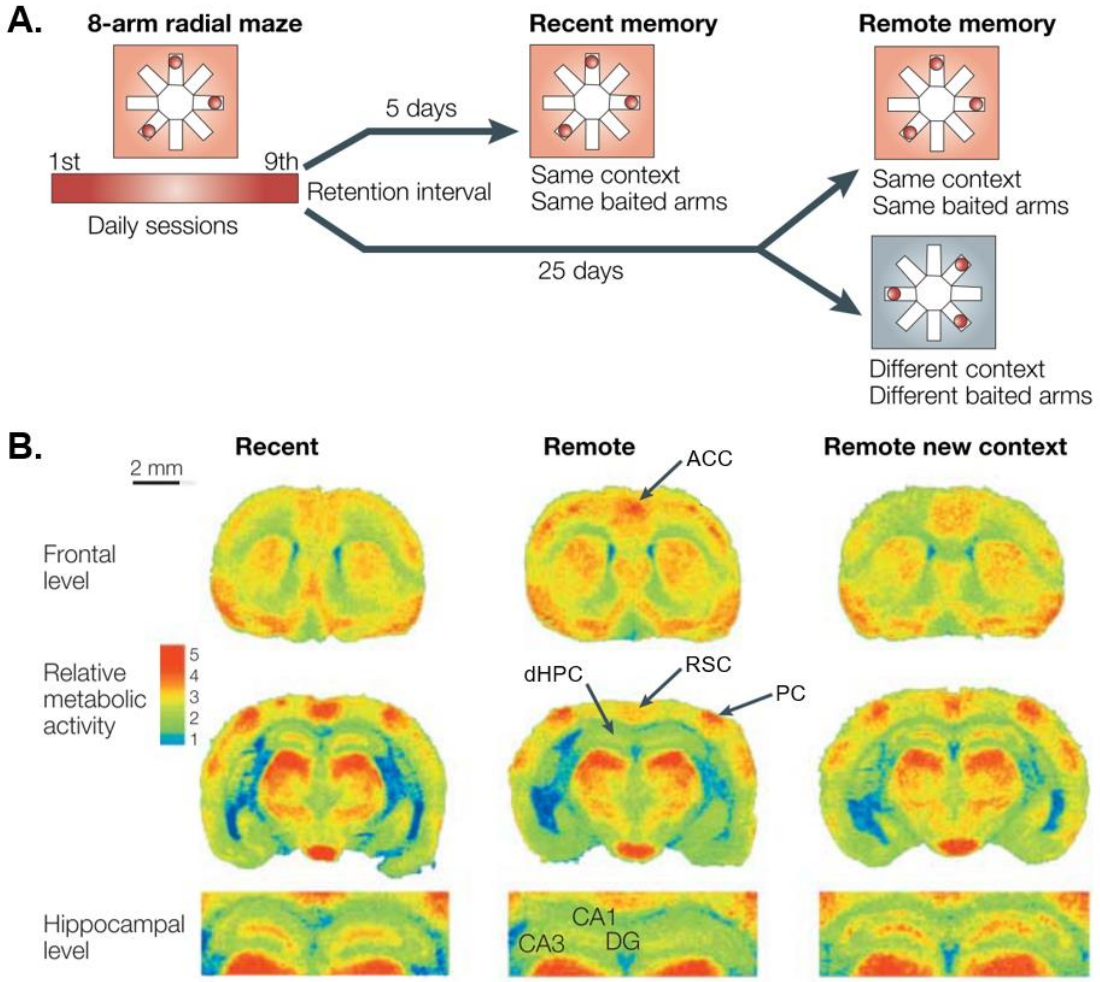

Figure 4.4. **A.** Behavioural protocol for training and recent/remote retention testing in the 8-arm radial maze used in Bontempi et al. (1999). **B.** Colour-coded autoradiographs following recent (left), remote (middle) and remote new context (right) testing. Adapted from Frankland and Bontempi (2005). ACC = anterior cingulate cortex; CA1 = Cornu Ammonis Area 1; CA3 = Cornu Ammonis Area 3; dHPC = dorsal hippocampus; DG = dentate gyrus; PC = parietal cortex; RSC = retrosplenial cortex.

Maviel, Durkin, Menzaghi, and Bontempi (2004) examined c-Fos instead of the metabolic markers using similar procedures to before when (^{14}C)2-deoxyglucose levels had been assessed. The benefit of this IEG was that they were able to look at cellular responses and determine whether different layers in the cortex responded differently at the recent and remote recall tests. Here, mice were tested for recall at days 1 or 30 following acquisition of the spatial memory task. An increase in cortical c-Fos only at day 30 was evident in the prefrontal and anterior cingulate cortices. This evidence suggests that these cortical regions have a more

prominent role in remote, but not recent, retrieval of this spatial memory task. However, there was also evidence of lamina reorganisation in the parietal cortex following remote versus recent recall. These additional cortical changes may reflect the gradual strengthening of cortical-cortical mapping over time of the remote spatial memory. A decrease in Zif268 expression in the dorsal CA1 and CA3 from day 1 to day 30 supported a transitory role for the HPC in the spatial memory task. To further support this, they next infused lidocaine to silence neuronal activity in the dorsal HPC. Inactivation of the HPC prior to testing removed the ability for mice to recall recent memories. Infusions of lidocaine to the PFC or ACC, conversely, impaired remote recall of the spatial task. The recruitment of cortical areas reported in this experiment may reflect the integrative and evolving roles of these areas in memory storage and recall of consolidated remote memories (Maviel et al., 2004).

Measuring IEG levels in both intact and ATN lesioned animals across both hippocampal and cortical regions may offer further insight into the impact that these lesions have on systems level consolidation. This was addressed in the context of a non-spatial, paired-associate odour-object memory task. The next section of this chapter therefore assesses association memory tasks that have been used previously following lesions to regions within the 'hippocampal-diencephalic-cingulate' network (Bubb et al., 2017). This thesis aimed to determine the role that the ATN has on acquisition of a non-spatial association memory task and, pending those finding, examine recent versus remote recall of that task after ATN lesions. We expect similar IEG activation patterns in intact rats that have been shown in previous spatial memory tasks; activation of the HPC at recent, and cortical regions at remote retention tests (Frankland & Bontempi, 2005; Maviel et al., 2004).

4.4 Association Memory Models of Amnesia

There is limited evidence of ATN involvement in explicit paired-associate learning tasks. This section therefore examines the effects of lesions to other regions in the 'hippocampal-diencephalic-cingulate' memory system, together with the evidence from ATN lesion studies (Bubb et al., 2017) (Table 4.3). Paired-associate memory explicitly requires the binding of arbitrary stimuli as a representation (i.e. place, odour, context, or object) that guides recollection (Eichenbaum & Fortin, 2009; Warburton & Brown, 2015). Animal models of these tasks aim to mimic aspects of everyday episodic memory in humans. Many tasks are "associative" in that memory reflects whether a set of stimuli have been encountered previously. Not surprisingly, lesions to regions in the 'hippocampal-diencephalic-cingulate' network (Bubb et al., 2017) generally produce deficits in spatial variations of associative memory and explicit paired-associate memory tasks.

Table 4.3. Summary of association memory studies with a focus on the 'hippocampal-diencephalic-cingulate' network (Bubb et al., 2017).

Author (Date)	Association Task, Apparatus	Behavioural Outcome	Lesion	Species
Sziklas and Petrides (1999)	**Object-place associative task** in an open field	ATN lesions impaired the acquisition of the spatial object-place associative task	ATN	Rat (hooded)
Sziklas and Petrides (2002)	**Object-place associative task** in an elevated plus maze	HPC but not Fx lesioned rats were impaired in the acquisition of the visual-spatial associative task	HPC; Fx	Rat (Hooded)
Gilbert and Kesner (2002)	**Odour/object-place paired associate tasks** in a cheeseboard apparatus 1. object-place paired associate task 2. odour-place paired associate task 3. object-odour paired associate task	HPC lesions impaired acquisition of spatially paired associations but not non-spatial associations 1 & 2. HPC lesions impaired acquisition 3. No acquisition impairment	HPC	Rat (Long-Evans)
Gilbert and Kesner (2003)	**Object/odour-place paired associate tasks** in a cheeseboard apparatus 1. object-place paired associate task 2. odour-place paired associate task	CA3 lesions impaired acquisition of both object-place and odour-place paired associations	DG; CA1; CA3	Rat (Long-Evans)
Henry et al. (2004)	**Object-place associative learning task** in an open field	ATN and HPC lesions impair acquisition of the spatial-visual associative learning task	ATN+HPC contralateral	Rat (Hooded)
Kesner et al. (2005)	**Object-trace-odour paired associate task** in a runway/box apparatus	CA1 but not CA3 required for the acquisition of the odour-trace-object paired association	CA1; CA3	Rat (Long-Evans)
Gibb et al. (2006)	**Odour-place paired associate task** in a cheeseboard apparatus	ATN and LT but not MT lesions required for the acquisition of the odour-place paired association	ATN; LT; MT	Rat (female hooded)
Hunsaker, Thorup, Welch, and Kesner (2006)	**Object-trace-place paired associate task** in a cheeseboard apparatus	With trace (temporal) and place (spatial) both CA1 and CA3 are required to acquire the paired-associate task	CA1; CA3	Rat (Long-Evans)

Author (Date)	Association Task, Apparatus	Behavioural Outcome	Lesion	Species
Barker, Bird, Alexander, and Warburton (2007)	**Object-in-place associative memory task** spontaneous association in an open field	PRh and mPFC disconnection (bilateral and contralateral lesions) impaired behaviour in the object-in-place associative task	mPFC, PRh: bilateral; ipsilateral; contralateral	Rat (Dark Agouti)
Rogers and Kesner (2007)	**Object-place paired associate task** in a cheeseboard apparatus	HPCxPCx contralateral lesions impaired acquisition of the object-place paired associate task	HPC+PC ipsilateral; contralateral	Rat (Long-Evans)
Barker and Warburton (2008)	**Object-in-place associative memory task** spontaneous association in an open field	Disruption of glutamatergic neurotransmission in the PRh and mPFC interrupted acquisition and retrieval of the object-in-place associative task	mPFC, PRh: bilateral; ipsilateral; contralateral	Rat (Dark Agouti)
Lee and Solivan (2008)	**Spatial paired associate** in a radial maze 1. object-in-place 2. location-in-place	HPC and mPFC required for the acquisition of an object-place paired-association. 1. HPC and mPFC lesions impaired acquisition 2. HPC lesions impaired acquisition	HPC; mPFC	Rat (Long-Evans)
Langston and Wood (2009)	**Object-place-context associative tasks** spontaneous association in an open field 1. object-place associative task 2. object-context associative task 3. object-place-context associative task	Large HPC lesions impaired the object-place-context associative task, but not the object-place or object-context associative tasks	HPC	Rat (Lister Hooded)
St-Laurent, Petrides, and Sziklas (2009)	**Object-place associative task** in an open field	Anterior and posterior cingulate lesions did not impair acquisition of the spatial-visual associative task, complete cingulate lesions gave rise to minor impairment	ACC, RSC	Rat (Long-Evans)
Jo and Lee (2010)	**Object-place paired associate task** in a radial maze	HPC, PRh and contralateral HPCxPRh disconnections severely impaired performance in the object-place paired association	HPC, PRh: bilateral; ipsilateral; contralateral	Rat (Long-Evans)

Author (Date)	Association Task, Apparatus	Behavioural Outcome	Lesion	Species
Cross, Brown, Aggleton, and Warburton (2013)	**Object-in-place associative memory task** spontaneous association in an open field	Bilateral and contralateral MD and mPFC lesions impaired acquisition of the object-in-place associative memory task	MD; mPFC: bilateral; ipsilateral; contralateral	Rat (Dark Agouti)
Dumont et al. (2014)	**Spatial/context discrimination tasks** operant chambers and field/box apparatus 1. auditory-visual/thermal discrimination 2. object/digging media-spatial discrimination 3. object/digging media-context discrimination 4. context-place discrimination	ATN lesions produced selective, severe deficits on a biconditional learning task using distal spatial cues (2)	ATN	Rat (Lister Hooded)
Dumont, Amin, Wright, Dillingham, and Aggleton (2015)	**Spatial/context discrimination tasks** field/box apparatus 1. object/digging media-context discrimination 2. object/digging media-spatial discrimination	Fx lesions delayed acquisition of a spatial biconditional task (2)	Fx	Rat (Lister Hooded)
Barker and Warburton (2018)	**Object-in-place associative memory task** spontaneous association in an open field	Permanent Re/Rh lesions impaired long-term memory; temporary lesions introduced in the encoding and retrieval stages impaired long-term memory of the object-in-place associative memory task	Re/Rh	Rat (Lister Hooded)

ACC = anterior cingulate cortex; ATN = anterior thalamic nuclei; CA1 = cornu ammonis 1 of the hippocampus; CA3 = cornu ammonis 3 of the hippocampus; DG = dentate gyrus of the hippocampus; Fx = fornix; HPC = hippocampus; LT = lateral thalamic aggregate; mPFC = medial prefrontal cortex; MD = mediodorsal thalamic nuclei; MT = posteromedial thalamic aggregate; PC = parietal cortex; PRh = perirhinal cortex; Re = nucleus reunions; Rh = rhomboid nucleus; RSC = retrosplenial cortex

Several studies have used evidence from odour-place and object-place paired-associate tasks to determine the role of brain regions in the encoding and recall of associations between arbitrary stimuli. These tasks require the ability to remember the specific locations in which objects/odours are embedded. They are the most common variation of paired-associate task in the literature. Ability to acquire such paired-associate memory has been tested in a range of apparatus, including the open field, a 'cheeseboard' and the radial maze. Acquisition impairments have been reported for both object- and odour-place paired associations following lesions of the ATN (Dumont et al., 2014; Gibb et al., 2006; Sziklas & Petrides, 1999) and the HPC (Gilbert & Kesner, 2002, 2003; Jo & Lee, 2010; Lee & Solivan, 2008; Sziklas & Petrides, 2002). There is also evidence that mPFC lesions produce deficits in object-place association (Lee & Solivan, 2008).

The spontaneous acquisition of an object-in-place is a variation of the object-place task that tests an animal's ability to acquire an association over one or more brief test sessions. There is a sample phase and a test phase. In the sample phase, the rat is commonly presented with four objects, one in each corner of an open-field, and allowed a certain amount of time to investigate the objects. In the test phase, the rat is presented with the same four objects but two of these have swapped their position within the arena. The ability to discriminate between the objects that have been previously associated with a location and those in a new location is measured. Despite both the objects and locations being individually familiar, the object-in-place task measures the association between these familiar objects, i.e. their combination within the apparatus (Aggleton & Nelson, 2020). Disconnection lesions to the PRh and mPFC (Barker et al., 2007; Barker & Warburton, 2008), MD and mPFC (Cross et al., 2013), and the nucleus reunions/rhomboid nucleus (Barker & Warburton, 2018) impair

rats' discrimination between objects in the previously encountered locations from objects in new locations.

Variations of the object-place/object-in-place association tasks have provided varying behavioural outcomes following lesions to the HPC. Sziklas and Petrides (2002) demonstrated that complete electrolytic lesions of the HPC in rats impaired acquisition, even with extensive training, of an object-place task. However, in the spontaneous object-in-place association task, rats with large bilateral ibotenic-acid lesions to the HPC were able to discriminate between the new and old locations of previously encountered objects (Langston & Wood, 2009). This would indicate that the HPC is recruited differently between the variations of object-place and object-in-place associative learning. For example, encoding of object-place associations across multiple trials depends on the integrity of the HPC, while spontaneous object-place associations do not (Langston & Wood, 2009). There has, however, been criticism over the variability in behavioural outcomes of the spontaneous object-in-place test. This comes from a range of factors, such as the objects used, the subjective nature of behavioural scoring by experimenters, inter-animal differences and the context within the field/apparatus being used (Aggleton & Nelson, 2020). These variations increase difficulty when interpreting behavioural results and determining the lesion effects (Albasser et al., 2010).

Disconnection crossed-lesion studies provide further evidence for associative learning deficits following lesions within the 'hippocampal-diencephalic-cingulate' network (Bubb et al., 2017). Disconnection studies disrupt communication between two structures by administering unilateral lesions to structures of interest in contralateral hemispheres (opposite), whereas the control comparison is to make ipsilateral lesions in the same

hemisphere. Contralateral lesions to the ATN and HPC in rats produced impairments in the acquisition of the spatial-visual associative task, of learning which of two objects was correct depending on the spatial context (Henry et al., 2004). Contralateral, but not ipsilateral lesions to the MD and mPFC (Cross et al., 2013), and the PRh and HPC (Jo & Lee, 2010), also produced severe impairments on behaviour for object-in-place associative learning. These disconnection studies provide evidence that the connections between the MD and mPFC as well as the PRh and HPC are critical for the associative discrimination of objects in spatial contexts. Contralateral disconnections of the PRh and mPFC also impair performance in spontaneous object-in-place associative learning task (Barker et al., 2007), with follow-up evidence that contralateral NMDA plasticity in these regions is critical for long-term but not short-term acquisition of this task (Barker & Warburton, 2008). Evidence from disconnection studies adds to the notion that there is a system of structures critical to mnemonic function beyond the hippocampus alone.

Dumont et al. (2014) examined the role of the ATN on spatial and non-spatial biconditional/paired-associate learning tasks that had previously been associated with HPC integrity. In the operant box, rats had to learn the non-spatial association between an auditory cue and the visual or thermal context in which the cue was presented. By contrast, explicit object-spatial and object-context associations were acquired in open fields. The object-spatial tasks required rats to learn the association between the digging media (object) and distal spatial cues. Object-context tasks required rats to form an association between digging media and the visual context (i.e. spot/check patterns) in the apparatus to receive a food reward. ATN lesions produced selective and severe deficit on the biconditional learning task requiring rats to use distal spatial cues, but spared behaviour on the auditory/visual-context, object-context, and context-place tasks. The results from these behavioural experiments

reflect those seen in rats with HPC lesions highlighting the interdependencies of the ATN and HPC.

The HPC has consistently been implicated in encoding of associations between objects or odours and the spatial locations in which they occur, but generally not associations in non-spatial contexts (Cho & Kesner, 1995; Gilbert & Kesner, 2002). Gilbert and Kesner (2002) trained rats with complete HPC lesions three paired-associate tasks: object-place, odour-place and object-odour. The presentation of stimuli in the object-odour task was simultaneous. In the object-place and odour-place tasks, the rats were trained to respond to object A (or odour A) when in location 1 but not location 2 and object B (or odour B) in location 2 and not location 1. Control rats were able to acquire these tasks over several weeks of training. HPC lesioned rats, however, did not show evidence of task acquisition when the task includes "place" during this time. In the non-spatial object-odour paired-associate task, rats were required to learn that odour X would be rewarded only when presented with object 1 and that odour Y would be rewarded only with object 2. HPC lesioned rats were able to acquire the non-spatial paired-associate task as well as controls. This evidence suggests that the HPC may be involved in associative learning only when one element of the pairing includes spatial location.

To determine the subregions within the HPC that are implicated in these tasks, Gilbert and Kesner (2003) tested rats on the object-place and odour-place tasks following selective dorsal HPC lesions to the DG, CA1 and CA3. Lesions to the DG and CA1 did not interrupt task acquisition, with these rats acquiring the task at a rate comparable to controls. Lesions of the dorsal CA3, however, removed the ability to acquire the odour-place and slowed down acquisition in the object-place spatial paired-associate tasks. As the CA1 is the primary

output of the HPC to the subiculum and entorhinal cortex, it was surprising that CA1 lesions did not impair acquisition on a spatial paired-associate task (Gilbert & Kesner, 2003; Langston, Stevenson, Wilson, Saunders, & Wood, 2010). It can be concluded that the dorsal CA3 is critical for acquisition of a spatial paired-associate task. However, it is important to note that ventral HPC connectivity was left intact in the lesion effects described above.

In another experiment, Kesner et al. (2005) investigated which HPC regions facilitate learning of non-spatial paired-associations across time. Here, an object-trace-odour paired-associate task was used. Rats were required to learn non-spatial associations between objects and odours when there was a 10 second 'trace' (delay) introduced between stimulus presentations. CA1 lesions removed the ability to acquire the association across the temporal lag, while CA3 lesioned rats learnt at the same rate as controls. This study provides evidence that, even for non-spatial stimuli, the HPC, specifically the CA1 is involved in forming arbitrary associations when there is a temporal element in the presentation of stimuli. In a later study, Hunsaker et al. (2006) combined objects with a spatial element in a temporal object-trace-place paired-associate task. Compared to the non-spatial object-trace-odour task, both lesions to the CA1 and CA3 completely removed the ability to acquire the object-trace-place task.

Taken together, these studies suggest that the CA1 is critical for solving paired-associate memory tasks that include a temporal component, whereas the CA3 is critical for solving tasks that include a spatial component. However, acquisition of a paired-associate task combining both spatial and temporal elements requires both CA1 and CA3 subregions of the HPC to be intact. This evidence suggests that injury to other regions within the 'hippocampal-diencephalic-cingulate' network (Bubb et al., 2017) may also produce deficits

in variations of these tasks. In the current thesis, the object-odour (Gilbert & Kesner, 2002) and object-trace-odour (Kesner et al., 2005) paradigms were used as the basis for the development of a task to determine brain activation in intact rats following recent and remote long-term recall when a temporal lag was included. Specifically, we hypothesise that a hippocampal-cortical shift of neural activation will be evident after remote testing for non-spatial paired-associate memory (Chapter 5). In Chapter 6, when tested for recent memory after reaching criterion, we hypothesise that there will be greater involvement of the dorsal CA1 in the trace non-spatial paired-associate task than the no-trace version.

Animal evidence highlights the need to look beyond the HPC to gain a better understanding of the neural networks underlying learning and memory. It is clear that spatial memory is interrupted following lesions to regions within the 'hippocampal-diencephalic-cingulate' network (Bubb et al., 2017). Impairment on paired-associate tasks with spatial elements following ATN lesions may be attributed to the fact that these lesions remove the ability to use space to guide behaviour rather than the inability to learn the specific association. The inability to learn a temporal order task that does not include any spatial element (Dumont & Aggleton, 2013; Wolff et al., 2006) indicates that the ATN may have a role in non-spatial memory. This thesis, therefore, examined the effects of ATN lesions in the non-spatial paired-associate task, with and without an additional temporal delay between the presentations of stimuli (Chapter 7).

4.5 Concluding remarks

Clinical and animal evidence both emphasise the need to look beyond the hippocampus in order to gain a better understanding of the neural regions associated with memory. Consistent evidence details significant memory deficits following injury to (or dysfunction in) regions

within the diencephalon and in particular the ATN. It is also clear that ATN injury leads to functional alterations in the 'hippocampal-diencephalic-cingulate' network. These alterations seem likely to contribute to behavioural deficits, in particular, IEG downregulation in the RSC. Non-human research into contribution of the ATN in memory is typically limited to spatial memory tasks. The HPC and diencephalon appear to function interdependently in spatial memory tasks. There is evidence that ATN lesions also impair memory for non-spatial information in temporal order tasks, which are also dependent on intact HPC functioning. More evidence is needed, however, that the ATN are involved in more than just spatial memory processing and recall. Non-spatial paired-associate learning provides one approach to address this issue.

In subsequent chapters (5-7) a novel non-spatial odour-object paired-associate task was tested in intact and ATN lesioned rats. In Chapter 5 the non-spatial task was tested across a temporal lag (odour-trace-object) in intact rats. The aim in Chapter 5 was to focus on a comparison of recent recall (at 5 days post-acquisition) and remote recall (at 25 days) to investigate neural activation following consolidation of the paired-associate task. Evidence from spatial memory tasks (Bontempi et al., 1999; Maviel et al., 2004) indicated that there would be a shift from hippocampal-activation to cortical-activation over time. The neural activation marker Zif268 was used here to focus on hippocampal subregions and the prefrontal and cingulate cortices in the trace paired-associate task. Chapter 6 compared IEG activation in intact rats following acquisition and recent recall (at 5 days only) of a No Trace and Trace variations of the odour-object paired-associate task. As above, we predicted that the dorsal CA1 would show greater activation following retention of an odour-trace-object paired-association due to the inclusion of a temporal lag (10 second delay) between stimuli presentation. This preliminary work was to establish the system-wide changes in Zif268

associated with the paired-associate task. Chapter 7 compared acquisition of the odour-object and odour-trace object paired-associate tasks in Sham and ATN lesioned rats. The question was whether ATN lesions impair either variant of this task. It is possible that ATN lesions would leave intact performance only when no trace (i.e. no temporal) component was used; if so, then the non-trace task could be used to assess consolidation effects after ATN lesions in a future study. IEG neural activation was also measured in both lesion groups following acquisition and recall of the No Trace and Trace conditions of the paired-associate task.

Chapter 5.

IEG response of a novel non-spatial paired-associate memory task: Recent vs remote memory

5.1 Introduction

Memory consolidation, as described in Chapter 4, is the time-dependent shift of information into enduring long-term memories (Dudai, 2004; Squire, 1992). The hippocampus (HPC) is critical in the formation of new memories, however, its role in consolidation is thought to be time-limited. The dominant view is that, over time, neocortical brain regions begin to mediate the retrieval of remote memories (Bontempi et al., 1999; Eichenbaum, 2000; Squire, 1992). Assessing immediate early gene (IEG) expression following recent and remote memory recall may reveal which neural regions are recruited during recall. IEGs, such as Zif268, are transcription factors that are rapidly expressed after cell activation in response to learning and memory (Guzowski et al., 2001; Hall et al., 2001). Regional expression of IEG proteins has also been used in studies on memory consolidation.

In two spatial memory studies, previously discussed in Chapter 4, the Bontempi Lab examined neural activation for recent and remote recall. Bontempi et al. (1999) reported a transitory role for the HPC in memory storage (recent recall) through mapping the metabolic marker (^{14}C)2-deoxyglucose. By contrast, some cortical sites were associated with remote spatial memory recall. The 30-day interval between training and retention of correct arm locations (the remote recall group) was associated with low metabolic levels in the HPC but high levels in prefrontal and anterior cingulate cortices. Like recall at 1-day, new learning at the "remote" time-point but involving new learning (new reward locations) revealed higher activation in the HPC, indicating that it is a critical region for encoding of new information

and the recent recall of spatial information (Bontempi et al., 1999). A second consolidation study, however, revealed changes in expression of the IEG, c-Fos, across cortical and hippocampal regions that also differed as a function of recent and remote recall (Maviel et al., 2004). IEG expression revealed a hippocampal to cortical shift from new learning and recent recall relative to remote recall. Moreover, IEG changes also uncovered a time-dependent shift in laminar organisation of the parietal cortex's neuronal activity during consolidation of remote memory. That is, the benefit of using an IEG as a marker for recall was to confirm a key theoretical process associated with cortical involvement in consolidation, namely functional cortical reorganisation.

We have also earlier seen that a fundamental aspect of episodic memory is the ability to bind arbitrary elements to form representations of the various stimulus components of an episode (Preston & Eichenbaum, 2013). Paired-associate tasks explicitly test this ability to form arbitrary associations and are feasible to use in animal models of episodic-like memory processes (Langston et al., 2010). As discussed in Chapter 4, there is clear evidence that the HPC and ATN are involved in spatial variants of paired-associate memory tasks (object-place or odour-place) (Gibb et al., 2006; Gilbert & Kesner, 2002, 2003; Kesner et al., 2002; Kesner et al., 2005; Sziklas & Petrides, 1999). Nonetheless, an important aspect of the key idea behind arbitrary paired-associate learning is that the deficit is not explained by a dominant impairment in processing any one of the components alone; this is particularly the case when "space" is one of the factors for solving the paired-associate task. For lesions that affect the hippocampal system and related neural structures, it is more informative to learn the neurobiological correlates associated with non-spatial paired-associate tasks.

Beginning with intact animals rather than lesion effects, however, one might expect to see a pattern of neural changes that reflect recall of the task. With respect to consolidation, we would anticipate that the pattern of neural changes would vary as a function of whether recall is recent or remote. Hippocampal lesions, and specifically CA1 lesions, are relevant for non-spatial paired-associate tasks that include a temporal component (Kesner et al., 2005). The temporal component used previously is a 10 second delay ("trace") between an object and an odour. The current study used a modified version of the object-trace-odour task developed by Kesner and colleagues. This object-trace-odour paired-associate task required rats to form arbitrary associations when stimuli were presented separately. This paired-associate task required rats to first interact with object and then decide whether to dig for a food reward in an odourised digging medium following a 10 second trace. The key difference from this task is that we reversed the order of presentation of stimuli. That is, the rat first responded to an odour, and then to an object after a 10 second delay. This enabled more direct responding by avoiding the need to dig for a reward; the rats made a nose-poke in an in an odourised sponge for a reward and then tipped over a hinged object for a second reward if the odour-object pairing was correct but should avoid responding to the object if the pairing was incorrect.

Using this adapted task, the current study examined neural activation following recent and remote recall of a paired-associate task with an inter-stimulus delay of 10 seconds. Intact rats were trained on this odour-trace-object paired-associate task. Once acquired, IEG Zif268 expression was assessed after a retention test at 5 or 25 days later. In addition, a third group of rats were tested on novel stimuli pairings in the odour-trace-object paired-associate task, introduced at day 25 post-acquisition; IEG responses in this condition would be expected to be more similar to that shown by the 5-day retention group. We expected to find time-

dependent frontal cortex activation, and perhaps parietal cortex activation, with higher levels of Zif268 expression at remote recall of this temporal paired-associate task than for recent and new-learning conditions. Due to the trace aspect, we expected hippocampal activation, specifically in CA1, in the recent recall condition, but not for the remote recall condition.

5.2 Materials and Method

5.2.1 Subjects

Twenty-two 12-month old male Long-Evans rats were used. They were bred and housed in the University of Canterbury's Animal Facility. Eight of the twenty-two rats (Group T5) provided data both for this memory consolidation study and the No-Trace/Trace comparison (Chapter 6). Rats were housed in groups of 3 or 4 per standard Makrolon cage (48 x 28 x 22 cm) in humidity (50±5%) and temperature-controlled conditions (21±2°C). Behavioural testing occurred in the dark phase of the reversed 12-hour light-dark cycle (lights on at 8pm). For this, rats were food-restricted to support 85% of free-feeding body weight with water ad libitum. All procedures carried out were approved by the Animal Ethics Committee of the University of Canterbury (#2017/20R).

5.2.2 Apparatus

The memory task was conducted in a red Perspex runway (93cm x 26cm x 26cm). Vertically removable doors to allow access to different compartments (B, C, D) were placed at X, Y, and Z (Figure 5.1, **A**). The odour receptacle (6.5 x 6cm clear Perspex) slotted into a central holder 10cm from the base of Door Y and held a thin sponge (different sponge for different odours) with a 2 cm circular white plastic cap at its centre in which food reward was placed (Figure 5.1, **C**); Door Y was the end of Compartment B. New odours were made fresh each week, by mixing 20µl essential oil (Essential Oils of New Zealand) in 5ml sunflower oil and 8 drops (~1ml) of odour added to the sponge. Fresh gloves were worn when handling the

odours and replacing the sponge to minimise odour transfer. Light, visually distinct objects (Figure 5.1, **B**) were attached by a hinge to the top of a black wooden food receptacle (5 x 9 x 3cm), so that the rat could easily push the object back to inspect the central well for a food reward; the object was placed at the end of Compartment D. Objects and the runway apparatus were cleaned between each rat with a 5% ethanol solution to reduce any odours cues from the previous rat. Food rewards of 0.1gm chocolate drops (FoodFirst LTD. Auckland, NZ) were used in both the odour and object receptacles. A wooden frame, draped in black fabric enclosed all sides and the top of the apparatus to reduce visual and light cues in each testing session. The experimenter always sat in the same position (small gap in the curtain at the midline of the apparatus) across all tasks and conditions, so that there were no condition-specific cues that the rats could use to influence their behaviour. Objects and odours were prepared on a table to the right of the apparatus.

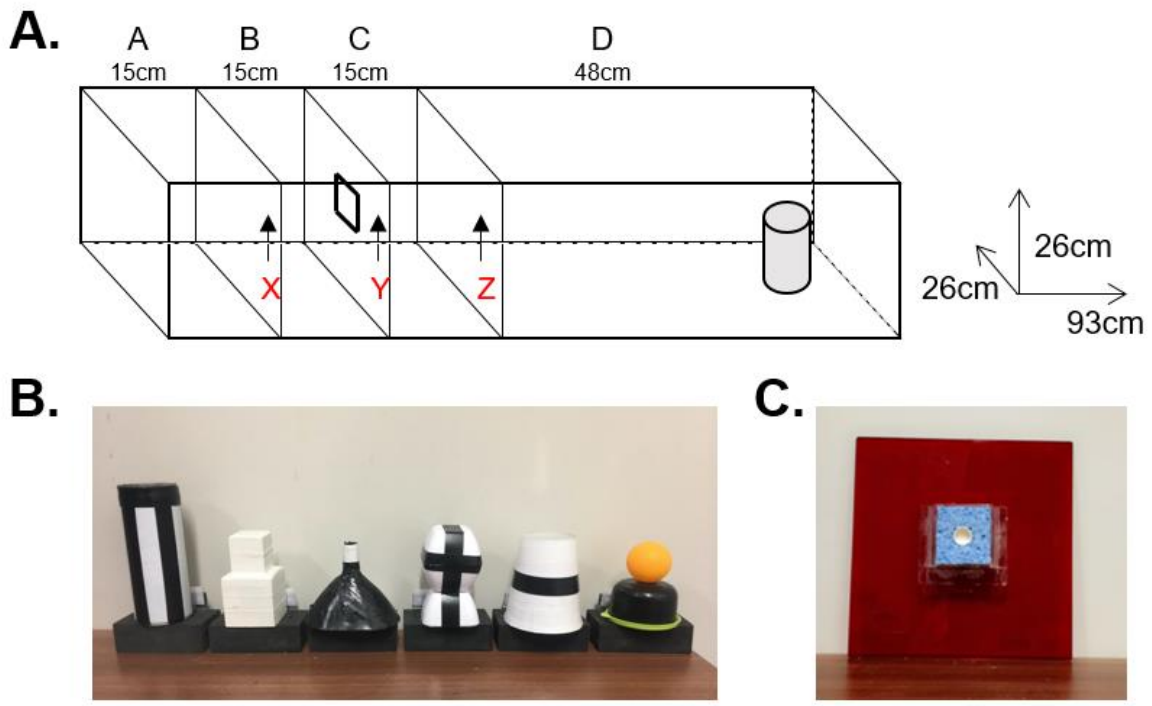

Figure 5.1. Schematic of the runway used to acquire the simple odour discrimination task, the simple object discrimination task, and the paired-associate memory task. **A.** Placement of doors for the **odour-trace-object paired-associate task**. Compartment A was used as the start box. The odour was presented on door Y at the end of compartment B. Compartment C was used as a delay/trace compartment. The far end of Compartment D presented the moveable object mounted by a hinge above the food receptacle. In the **simple odour and simple object discrimination tasks**, the rats started in compartment A with the odour or object presented at the end of compartment D; only door X was used and the door (Y) holding the odour was placed at the end compartment D and secured to the end wall with electrical tape. **B.** Objects used in both the simple discrimination task and the association memory tasks. **C.** Odour receptacle in the door used in both the simple discrimination task and the paired-associate memory tasks. Each odour had its own door to reduce the ability for odour transfer. The food reward was presented in the white cap at the centre of the sponge, which was infused with 8 (approx. 1ml total) drops of odour around the external edges.

5.3 Method

Over two weeks rats were food-restricted to reduce and maintain their body weight to 85% of ad libitum weight. They were handled for 5 minutes a day and habituated to the food reward in their home cages. On approaching the 85% weight target, they were habituated to the runway in cage groups and then singly 10 minutes. Rats were placed in the start box (compartment A) and chocolate (0.1g) was placed throughout the runway for three days to habituate to the apparatus. The following day, rats were then shaped to nose poke an

odourless sponge (for one chocolate drop) by using a single line of chocolate drops leading to the in the sponge on the door fixed to then end of compartment D (maximum 4 days). Rats were then shaped to push an object (an object not used subsequently) back to retrieve two chocolate food rewards (maximum 5 days). As with the odour, a line of chocolate encouraged the rat to approach the object at the end of the apparatus, already tipped, before the rat then had to push the object back to obtain the two chocolate drops.

Subsequently, half of the rats received training in the simple odour discrimination task followed by the simple object discrimination and vice versa for the other half. Rats were randomly assigned to one of three odour-trace-object conditions based on post-acquisition retention test at: 1) 5 days (T5, n=8); 2) 25 days (T25, n=8); or 3) a new association (novel odour-trace-object pairing) tested at 25 days after the end of acquisition of the initial task (T-New, n=6). All tasks used a 'go, no-go' procedure. The first trial on any day began by keeping the rat in the start area (compartment A; Figure 5.1) for 120 seconds to reacclimatise to the maze. For all tasks, rats received 12 massed trials per daily session, 6 'go' (correct pairing) and 6 'no-go' (incorrect pairings) trials in a pseudo-randomised order with no identical pairing run consecutively in the paired-associate task. No more than three consecutive 'go', or three 'no-go' trials were run consecutively in a session. For example, two 'go' trials could be run sequentially, but not of the identical pairing. Correct pairings in both simple discrimination and paired-associate tasks were counterbalance across rats. They were trained to reach criterion of ~80% correct trials for three consecutive days. A trial was designated as "correct" if the rat responded in less than 8 seconds on 'go' trials and not responding before 8 seconds in 'no-go' trials. For the odour, a response was defined as a nose poke into the cap in the centre of the sponge to receive a reward; sniffing the sponge but ignoring the food reward was considered a 'no-go' response. For the object, a response was

defined as any push that lifted the object with nose or paw. Latency between the door (Door X for simple discrimination; Door Z for paired-associate task) and interaction with the odour or object was recorded. Rats were trained on the simple odour and simple object discrimination task until they reached criterion (80% correct over 2 consecutive days).

5.3.1 Simple odour discrimination task

A single odour was presented on each trial. The rat had to discriminate which of two odours (i.e. either odour 1: lemon, or odour 2: clove) was paired with a food reward (one chocolate drop) presented in the sponge at the end of compartment D.

5.3.2 Simple object discrimination task

A single object was presented on each trial at the end of Compartment D. The rat had to learn which of two objects (i.e. either object A, black and white striped can; or object B, white square blocks) was paired with food reward (two chocolate drops) presented under the object at the end of compartment D.

5.3.3 Odour-trace-object paired-associate memory tasks

The rats learned which of two object-odour pairings would be rewarded and which two were non-rewarded. Irrespective of the pairing, rats always received a reward for the odour presented (one chocolate drop at the centre of the sponge), and then received a food reward (two chocolate drops) under Object C if presented after Odour 3, but not if presented with Object D after Odour 3. Conversely, Object D following Odour 4 would be rewarded but not Object C following Odour 4. The correct pairings were counterbalanced across rats. The trace, a 10-second delay in Compartment C (**A**, Figure 5.1), was introduced immediately following consumption of the chocolate reward in the cap in the centre of the odourised sponge. During the trace period, the object in Compartment D was obscured from view for

the rat by door Z. Latency was measured from the time Door Z was lifted until the rat pushed the object for the food reward or refrained from pushing the object for 8 seconds. The rats were trained on the odour-trace-object association task until they had reached criterion (80% correct trials over 3 days) or for a maximum of 43 days.

5.3.4 Post-acquisition test

A post-acquisition test was conducted, using 12 massed trials, either 5 or 25 days after the rat reached criterion or at the end of 43 days of formal training. This test consisted of the same previously learned association for Groups T5 (5 days post-acquisition) and T25 (25 days post-acquisition) or a completely new association i.e. Odours 5 and 6 and Objects E and F for Group T-New (tested on a new odour-trace-object paired-associate task at 25 days post-acquisition on the original task).

5.3.5 Histological procedures

Perfusion

Following the last trial of the post-acquisition test, rats were housed singly in a cage in a dark, quiet room for 90 minutes before perfusion so that Zif268 IEG protein expression in the brain would primarily reflect experience during the preceding memory testing. The rats had been habituated to this room for 3 days prior to the post-acquisition test. Rats were deeply anaesthetised with pentobarbital (125mg/kg). Once under deep anaesthesia and completely unresponsive to stimulation, the rat was perfused transcardially with heparinised saline followed by paraformaldehyde (PFA 4% in 0.1M phosphate buffer) to fix the brain. The perfused brains were post-fixed in PFA 4% overnight at 4˚C followed by a minimum of 48hrs in a long-term solution (20% glycerol in 0.1M PB). Coronal 40µm sections were collected on a freezing stage sliding microtome (Thermofisher, UK) and stored in cryo-protectant solution (30% glycerol, 30% ethylene glycol in 0.1M PB) at -20˚C for immunohistochemistry.

Zif268 immunohistochemistry

All sections and all groups for each ROI were processed in 6-well staining trays simultaneously (i.e. all rats processed at the same time). Free floating sections were washed three times in 0.1M phosphate buffered saline containing 0.2% Triton X-100 (PBSTx) for 10 minutes before being incubated in endogenous peroxidase blocking buffer for 30 minutes (1% hydrogen peroxide (H_2O_2), 50% methanol (CH_3OH) in 2% PBSTx). Excess blocking buffer was removed with three 10-minute PBSTx washes, before sections were incubated in rabbit polyclonal Zif268 primary antibody (Egr-1; 1:1000; Santa Cruz Bio) for 72 hours at 4˚C in PBSTx with 1% normal goat serum (NGS). Excess antibody was removed with three 10-minute washes in PBSTx followed by incubation in biotinylated goat anti-rabbit secondary antibody (1:1000: Vector) overnight in PBSTx and 1% NGS. Sections were washed three times (10 minutes each) in PBSTx before 2-hour incubation in ExtrAvidin (peroxidase conjugated; 1:1000; Sigma) in PBSTx and 1% NGS. To remove excess ExtrAvidin and Triton X-100, the sections were washed three times in phosphate buffered saline (PBS) and three times in phosphate buffer (PB). This was followed by two 5-minute washes of Tris buffer (Tris hydrochloride, pH 7.5), to prepare the sections for visualisation with diaminobenzidine (DAB). The DAB (0.05%; Sigma) with 0.01% H_2O_2 in Tris buffer reaction (approximately 20 minutes) was stopped using Tris buffer (1x 10 minute wash), followed by PB (1x 10 minute wash) and sections placed in PB at 4˚C overnight before mounting onto gelatinised slides and allowed to dry. The slides were dehydrated through graded alcohol (70-100%) before being cleared in xylene and cover slipped with DPX.

5.3.6 Regions of interest

Regions of interest (ROIs) were selected that had potential for involvement in the odour-trace-object paired-associate memory task and its consolidation (Figure 5.2). These regions

included the medial prefrontal cortex/cingulate cortex (A32D, A32V, A24a and A24b), anterior gustatory cortex (piriform and agranular insular cortices), hippocampus (dorsal: CA1, CA3 and dentate gyrus (DG); ventral: CA1 and CA3), subiculum (dorsal and ventral), parietal cortex, retrosplenial cortex, and the parahippocampal cortex (perirhinal and entorhinal cortices).

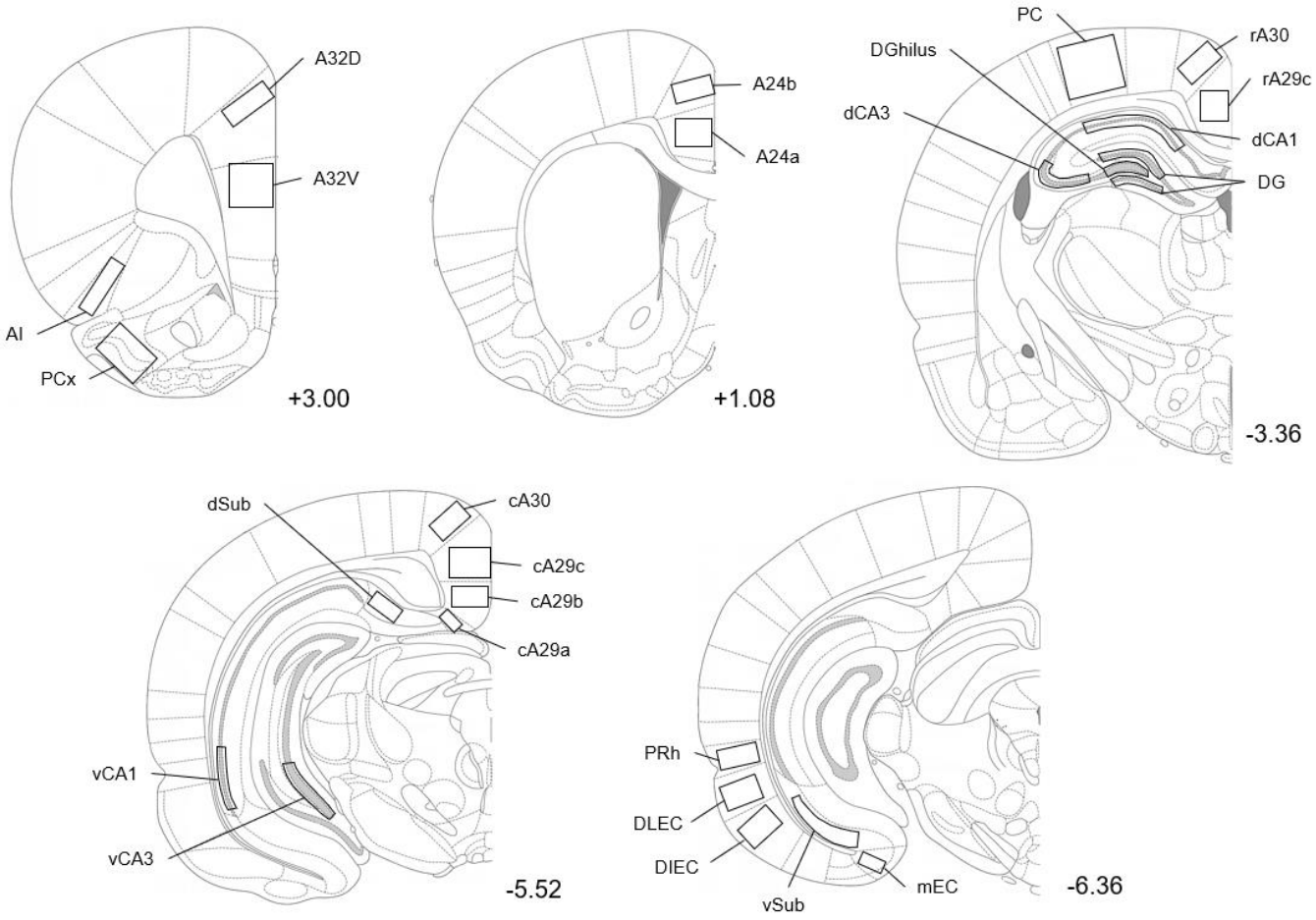

Figure 5.2. Regions of interest included in quantification of Zif268 expression. A24a/b = area 24a/b of the anterior cingulate cortex; A32D/V = dorsal/ventral area 32 of the anterior cingulate/prefrontal cortex; AI = agranular insular cortex; dCA1 = Cornu Ammonis Area 1 of the dorsal hippocampus; dCA3 = Cornu Ammonis Area 3 of the dorsal hippocampus; DG= dentate gyrus of the dorsal hippocampus; DGhilus = hilus of the dentate gyrus of the dorsal hippocampus; DIEC = dorsal intermediate entorhinal cortex; DLEC = dorsolateral entorhinal cortex; dSub = dorsal subiculum; mEC = medial entorhinal cortex; PC = piriform cortex; PRh = perirhinal cortex; r/cA29a/b/c = rostral/caudal area 29a/b/c of the granular retrosplenial cortex; r/cA30 = rostral/caudal area 30 of the retrosplenial cortex; vCA1 = Cornu Ammonis Area 1 of the ventral hippocampus; vCA3 = Cornu Ammonis Area 3 of the ventral hippocampus; vSub = ventral subiculum.

5.3.7 Zif268 Quantification

ROI sections showing Zif268-positive staining were identified and photographed at 10x objective with a light microscope (Leica, Germany). Automated counts of the cells were obtained through ImageJ (image analysis software, National Institute of Health, NIH, USA). The ROI was selected and the images were converted to 8-bit grey scale, background was

subtracted (rolling = 40), converted to mask and the watershed function was applied, and all Zif268 positive cells above threshold ('MaxEntropy' threshold, circularity 0.7-1) were counted. Counts of Zif268-positive cells for all regions used the same threshold algorithm, with between two to six sections per region of interest in each rat quantified. The average Zif268 positive cell count per mm^2 across sections within an ROI per rat was analysed.

5.3.8 Statistical analysis.

Statistical analyses were conducted using Statistica (v13; Dell Inc.). A reciprocal transformation of latency data for each individual trial was used to ensure homogeneity of variance. Each latency score (for each individual 'go' and 'no-go' trial) was transformed prior to any statistical manipulation. For each rat, the mean latency difference ('go' minus 'no-go' trial reciprocal latencies, per day) was used to assess performance in each simple discrimination task and the paired-associate task. Repeated measures ANOVA and one-way ANOVA were conducted to test group differences in task acquisition, trials to criterion and Zif268 counts within and between ROIs. Significant main effects and interactions were further examined with post-hoc comparisons and simple main effects. Significance was set at $p<0.05$.

5.4 Results

5.4.1 Simple discrimination

There were no differences between the three groups in acquisition for either the simple odour discrimination (Figure 5.3; Group main effect, $F(2,19)=0.406$, $p=0.67$; Day x Group interaction, $F(12,114)=1.01$, $p=0.43$) or the simple object discrimination (Group main effect, $F(2,19)=0.66$, $p=0.52$; Day x Group interaction, $F(12,114)=0.68$, $p=0.76$). Rats took an average of 4 to 5 days to learn the simple odour discrimination (Day main effect, $F(6,114)=18.00$, $p<0.001$; Group main effect for trials to criterion, $F2,19)=0.52$, $p=0.59$).

More variable performance was evident for acquisition of the simple object discrimination with 5 to 6 days taken to learn the task (Day main effect, $F(6,114)=11.24$, $p<0.001$; Group main effect for trials to criterion, $F(2,19)1.98$, $=0.16$).

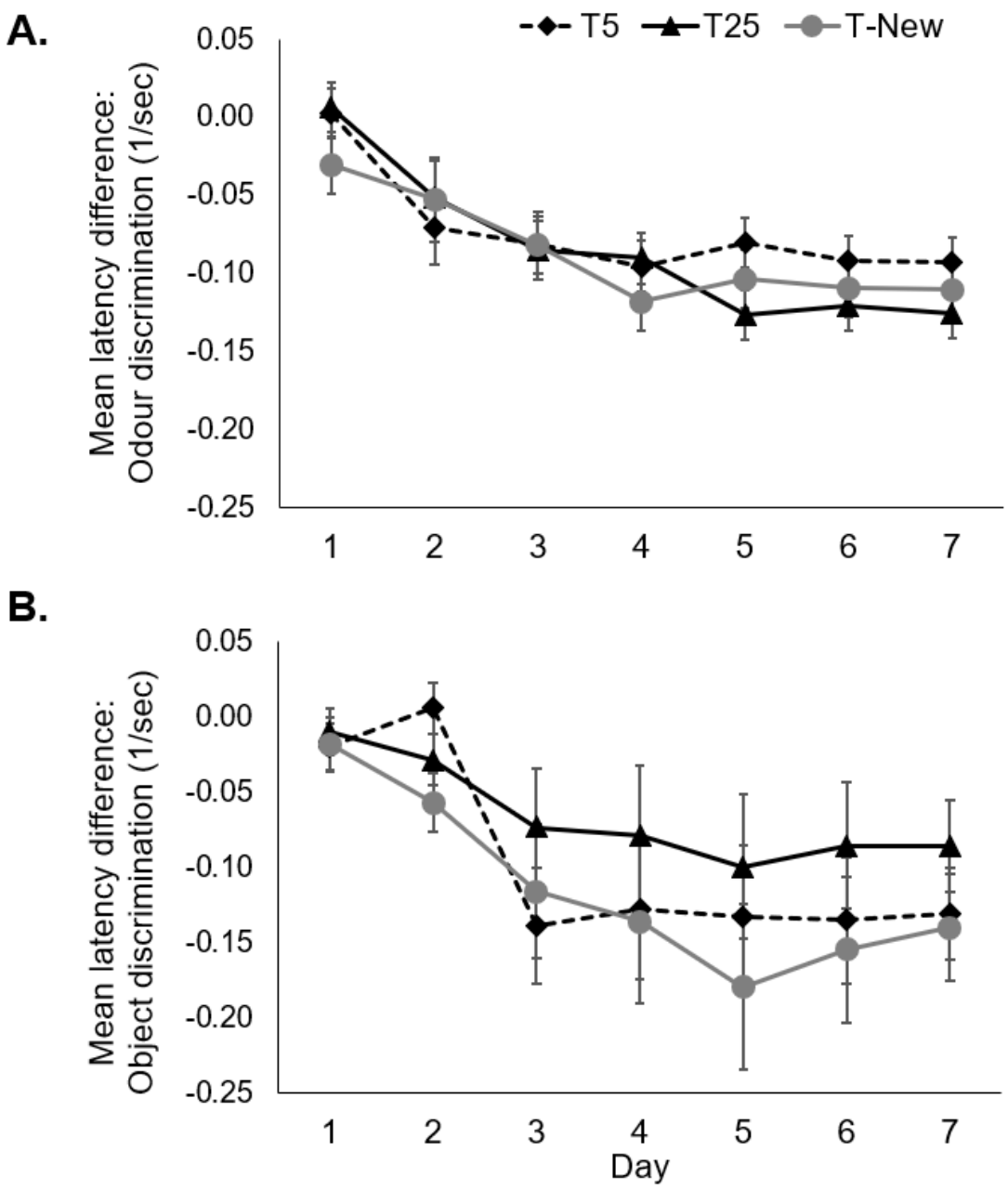

Figure 5.3. Acquisition (reciprocal mean latency difference) of the **A.** Simple odour discrimination and **B.** simple object discrimination tasks. 0.00 represents no difference in responding and -0.10 corresponds to approximately 3 second latency difference between 'go' and 'no-go' trials. Error bars = standard error of the mean. T5 = Trace Group, 5-day retention test after reaching acquisition criterion; T25 = Trace Group, 25-day retention test after

reaching acquisition criterion; T-New = Trace Group, new association task at 25-days after acquisition of the original odour-trace-object task.

5.4.2 Odour-trace-object paired-associate task acquisition and retention

All three groups of rats acquired the paired-associate task at the same rate (Group main effect for trials to criterion, $F(2,19)=0.40$, $p=0.67$). The first rat reached criterion at day 28 of training (Figure 5.4). Of the 22 rats, 14 acquired the odour-trace-object association within 43 days of testing, but 3 rats in each of the T5 and T25 groups and 2 rats in the T-New group did not reach criterion, despite showing clear latency differences between correct and incorrect trails. The final mean latency difference score was carried forward for those rats that had reached criterion prior to day 43. Latency measures shows that the three groups had similar rates of acquisition (Group main effect, $F(2,19)=0.97$, $p=0.39$; Group x Trial Block $F(20,190)=1.32$, $p=0.16$). Performance on the final block of testing did not differ significantly across groups (Group main effect $F(2,19)=2.60$, $p=0.09$).

Comparison of the last block of training confirmed the relative differences across the three groups at retention (Trial Block x Group $F(2,19)=36.48$, $p<0.001$; Figure 5.4). Group T5 did not change performance (simple main effect for T5, $F(1,19)=0.33$, $p=0.57$) whereas Group T25 showed a retained good discrimination of the task but a significant drop at retention ($F(1,19)=8.18$, $p=0.009$). As expected, Group T-New were unable to distinguish the new ‘go’ and ‘no-go’ pairings compared to discrimination scores of block 11 (simple main effect of Group T-New, $F(1,19)=128.25$, $p<0.001$). These differences gave rise to a significant group effect on the retention test alone ($F(2,19)=47.96$, $p<0.001$), confirming that Group T-New performed significantly worse than both T5 (simple main effect $F(1,19)=92.63$, $p<0.001$) and T25 ($F(1,19)=48.83$, $p<0.001$); Group T25 performed worse that Group T5 ($F(1,19)=8.10$, $p=0.01$).

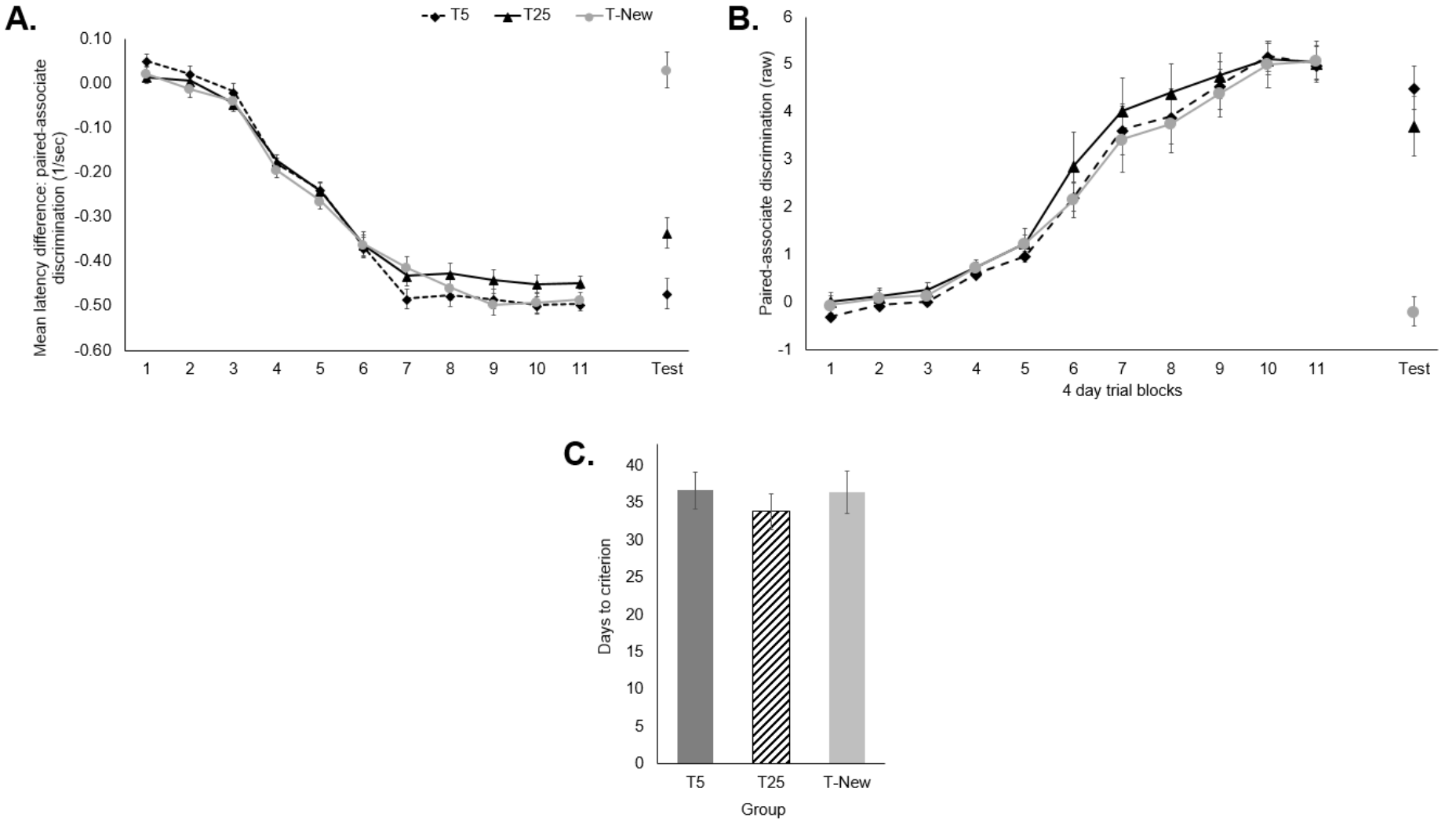

Figure 5.4. Acquisition and retention of the odour-trace-object association memory task in 4-day trial blocks. **A.** Normalised acquisition latency scores; mean latency difference of 0.00 represents no difference, and -0.50 represents approximately a 5 second difference in responding to the 'go' and 'no-go' trials. **B.** Raw mean latency difference discrimination scores in seconds. **C.** Average days to criterion for each group. Error bars = standard error. T5 = Trace Group, 5 day retention test after criterion (or maximum of 43 days); T25 = Trace Group, 25 day retention test after criterion (or maximum of 43 days); T-New = Trace Group, new association task at 25-days after acquisition of the original odour-trace-object task (or maximum of 43 days).

5.4.3 Zif268 IEG analysis

Zif268 counts were analysed across the ROIs comparing the three trace groups following the retention test. Significant differences were limited to the ventro-medial prefrontal cortex, retrosplenial cortex and the perirhinal cortex, so these are addressed first.

Prefrontal cortex – A32V

Expression of Zif268 in area 32V (A32V) of the prefrontal cortex following the retention test is shown in Figure 5.5. Collapsed across all layers of A32V, the three groups displayed similar Zif268 expression (Group main effect $F(2,19)=1.54$, $p=0.23$). While the groups did not differ for layers III, V and VI, remote recall of the previously acquired task at day 25 (Group T25) resulted in higher Zif268 expression in the superficial layer (II) of A32V (Layer x Group interaction $F(6,57)=3.60$, $p=0.004$). Post-hoc pairwise analysis for Layer II confirmed a higher count for group T25 than both the T5 ($F(1,19)=5.77$, $p=0.02$) and the T-New Groups ($F(1,19)=11.23$, $p=0.003$), which did not differ ($F(1,19)=1.26$, $p=0.27$). No group effects were evident in the other three layers.

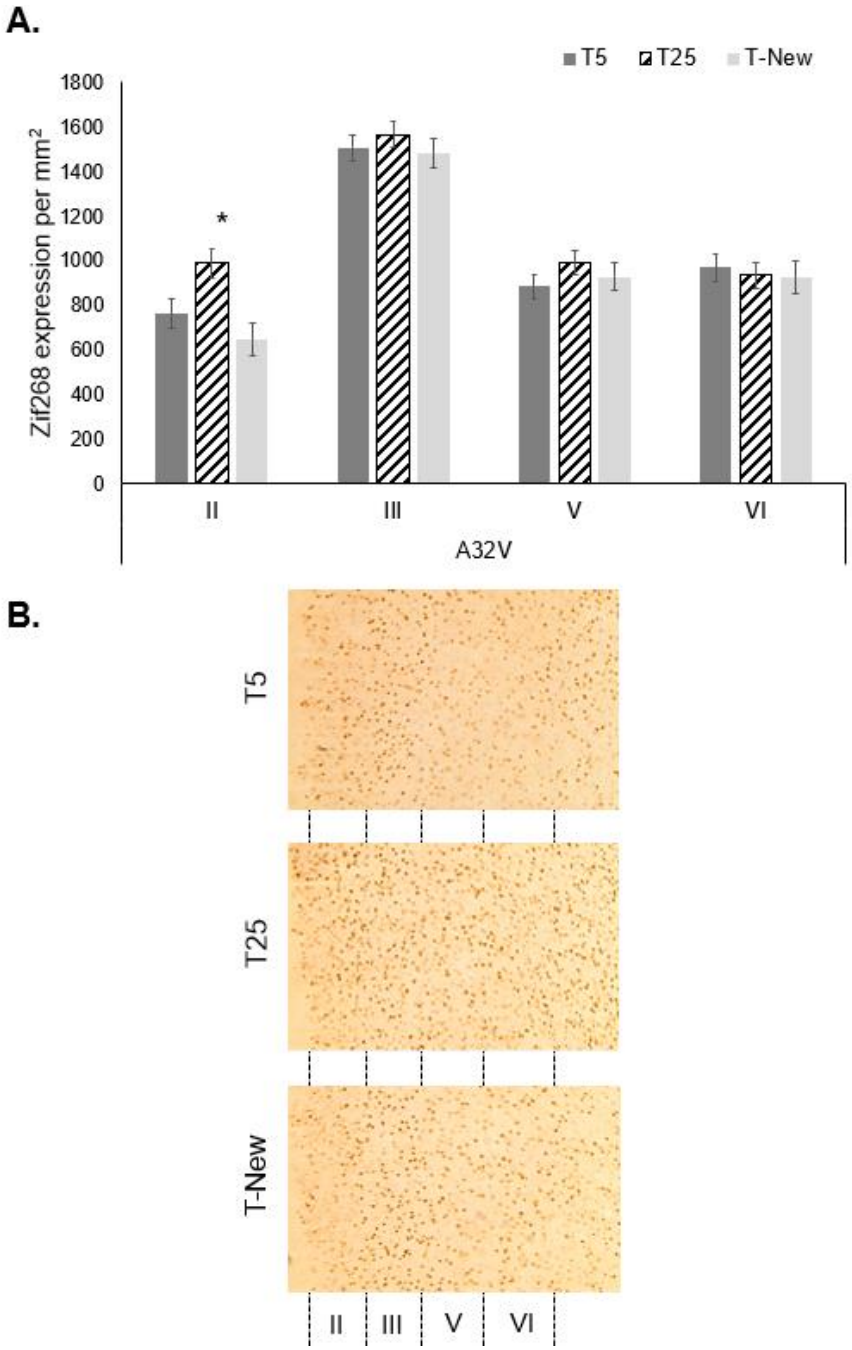

Figure 5.5. Zif268 expression in the layers of A32V of the prefrontal cortex in the three groups (Mean ± Std. Err). **A.** A32V Zif268 expression/mm^2 in the three trace groups: T5 (5-days post odour-trace-object task acquisition), T25 (25 days post odour-trace-object task acquisition) and T-New (new task presented 25 days after acquisition. Higher expression in the T25 group was found in layer II, *$p<0.05$ **B.** 10x magnification photomicrograph examples of Zif268 expression in the layers of area 32V of prefrontal cortex. Images represent the median in each group. II/III/V/VI = Layers of A32V;T5 = Trace Group, 5 day retention test after criterion (or maximum of 43 days); T25 = Trace Group, 25 day retention test after criterion (or maximum of 43 days); T-New = Trace Group, new association task at 25-days after acquisition of the original odour-trace-object task (or maximum of 43 days).

Retrosplenial cortex

Expression of Zif268 in rostral A29c (rA29c) and rostral A30 (rA30) regions of the RSC following the retention test is shown in Figure 5.6. Retention group T5and to a lesser extent Group T25 expressed more Zif268 in the rostral RSC compared to the T-New group (; Group main effect: rA29c $F(2,18)=6.74$, $p=0.006$; and rA30 $F(2,18)=10.90$, $p<0.001$). The highest counts were observed in Group T5 (simple main effect T5 vs T-New: rA29c $F(1,18)=13.22$, $p=0.001$; rA30 $F(1,18)=21.72$, $p<0.001$). To a lesser extent, Group T25 also expressed significantly higher counts than T-New (simple main effect T25 vs T-New: rA29c $F(1,18)=6.01$, $p=0.02$; A30 $F(1,18)=8.02$, $p=0.01$). T5 and T25, however, did not differ significantly from each other across both rostral RSC regions (simple main effect T5 vs T25: rA29c $F(1,18)=1.34$, $p=0.26$; rA30 $F(1,18)=3.30$, $p<0.08$). But there was no Group by Layer interaction for rA29c($F(2,18)=2.23$, $p=0.13$). There was however a Group by Layer interaction in rA30 ($F(2,18)=7.08$, $p=0.005$). Both retention groups (T5 and T25) expressed a higher number of cells in the superficial layer of rA30 than the T-New Group (simple main effect: T5 vs T-New, $F(1,18)=20.27$, $p<0.001$; T25 vs T-New $F(1,18)=9.04$, $p=0.007$) but did not differ from each other ($F(1,18)=2.14$, $p=0.160$). However, rats tested on the 5-day retention test (Group T5) showed higher Zif268 counts in the deep layer of rA30 than both other groups tested at 25 days (T25 $F(1,18)=4.56$, $p=0.046$; T-New $F(1,18)=13.63$, $p=0.001$), which did not differ from each other ($F(1,18)=2.55$, $p=0.12$).

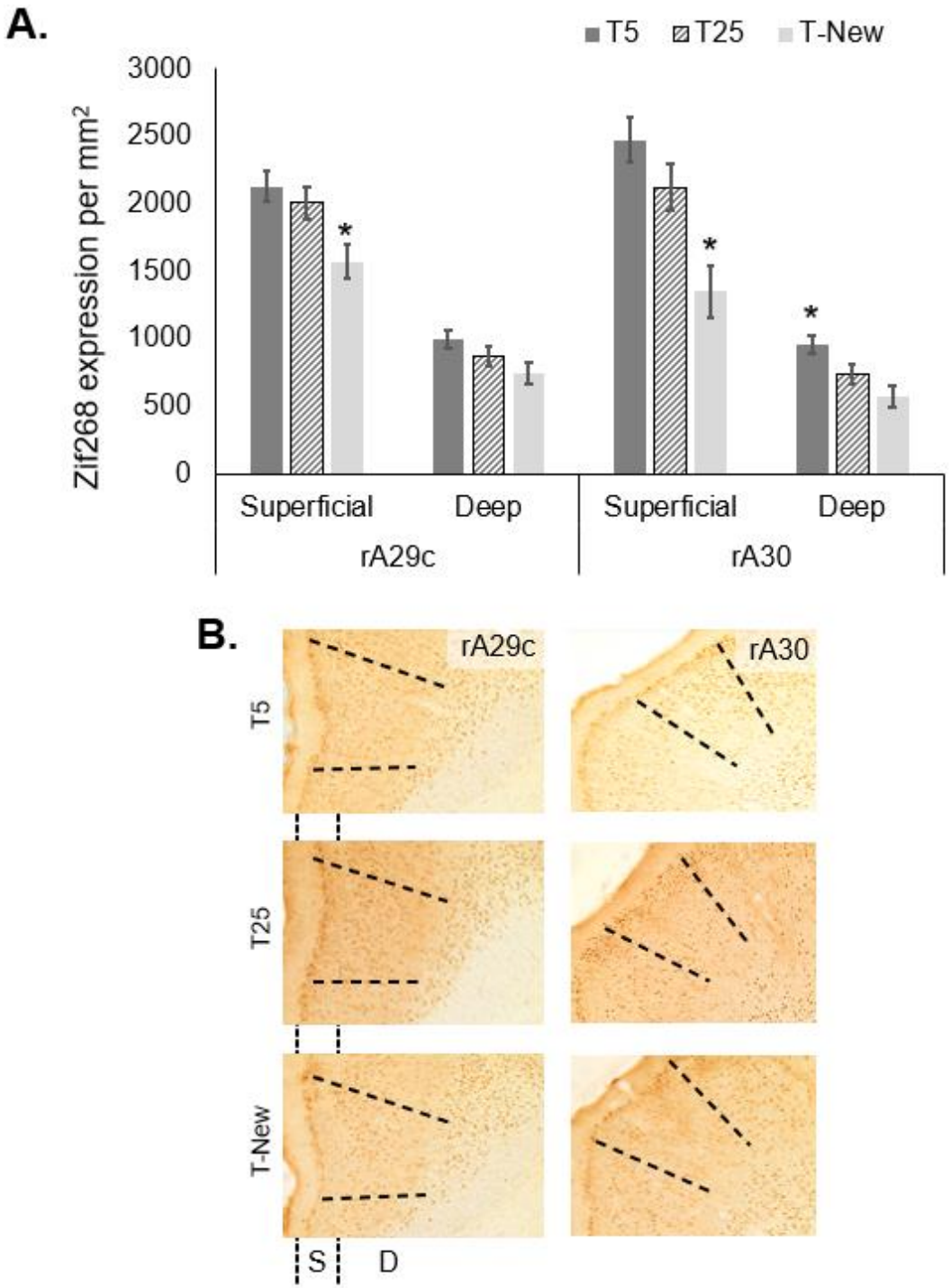

Figure 5.6. Zif268 expression in the superficial and deep layers of rostral A29c (rA29c) and rostral A30 (rA30) of the retrosplenial cortex following the retention test at 5- and 25-days post task acquisition. **A.** rA29c and rA30 Zif268 expression/mm^2 in the three groups: T5, T25 and T-New. Lower expression in Group T-New superficial rA29c and rA30, higher expression in T5 deep rA30, *$p<0.05$. **B.** 10x magnification photomicrograph examples of Zif268 expression in the layers of rA29c and rA30. Images represent the median in each group. D = deep layer; S = superficial layer; T5 = Trace Group, 5 day retention test after criterion (or maximum of 43 days); T25 = Trace Group, 25 day retention test after criterion (or maximum of 43 days); T-New = Trace Group, new association task at 25-days after acquisition of the original odour-trace-object task (or maximum of 43 days).

Expression of Zif268 in caudal A29 and A30 regions of the RSC following the 5- and 25-day retention test is shown in Figure 5.7. Although Group T-New appears to consistently have lower Zif268 expression across the caudal A29 regions this difference did not reach significance (Group main effect, $F(2,17)=2.96$, $p=0.078$). cA29c expressed higher levels of Zif268 than both cA29a and cA29b (Subregion main effect, $F(2,34)=8.62$, $p<0.001$; cA29c simple main effect: versus cA29a, $F(1,17)=6.06$, $p=0.02$; and versus cA29b, $F(1,17)=25.90$, $p<0.001$). cA29a and cA29b did not differ in expression ($F(1,17)=1.64$, $p=0.21$). The three groups showed similar patterns of cell counts across the cA29 regions (Subregion x Group interaction, $F(4,34)=0.09$, $p=0.98$). The superficial layer showed significantly higher counts of Zif268 than the deep layer (Layer main effect, $F(1,17)=453.04$, $p<0.001$). The most relevant finding was that Group T-New expressed lower levels of Zif268 in the superficial layers of cA29 than both retention groups (T5 and T25), but similar levels to these groups in the deep layer (Layer x Group interaction, $F(2,17)=6.93$, $p=0.006$; Superficial layer simple main effect: T-New vs T5, $F(1,17)=7.98$, $p=0.01$; T-New vs T25, $F(1,17)=7.64$, $p=0.01$; Deep layer simple main effect: T-New vs T5, $F(1,17)=0.09$, $p=0.76$; T-New vs T25, $F(1,17)=0.43$, $p=0.51$). There were no caudal A30 Group differences ($F(2,17)=0.49$, $p=0.61$) or Group by Layer interactions ($F(2,17)=0.76$, $p=0.48$).

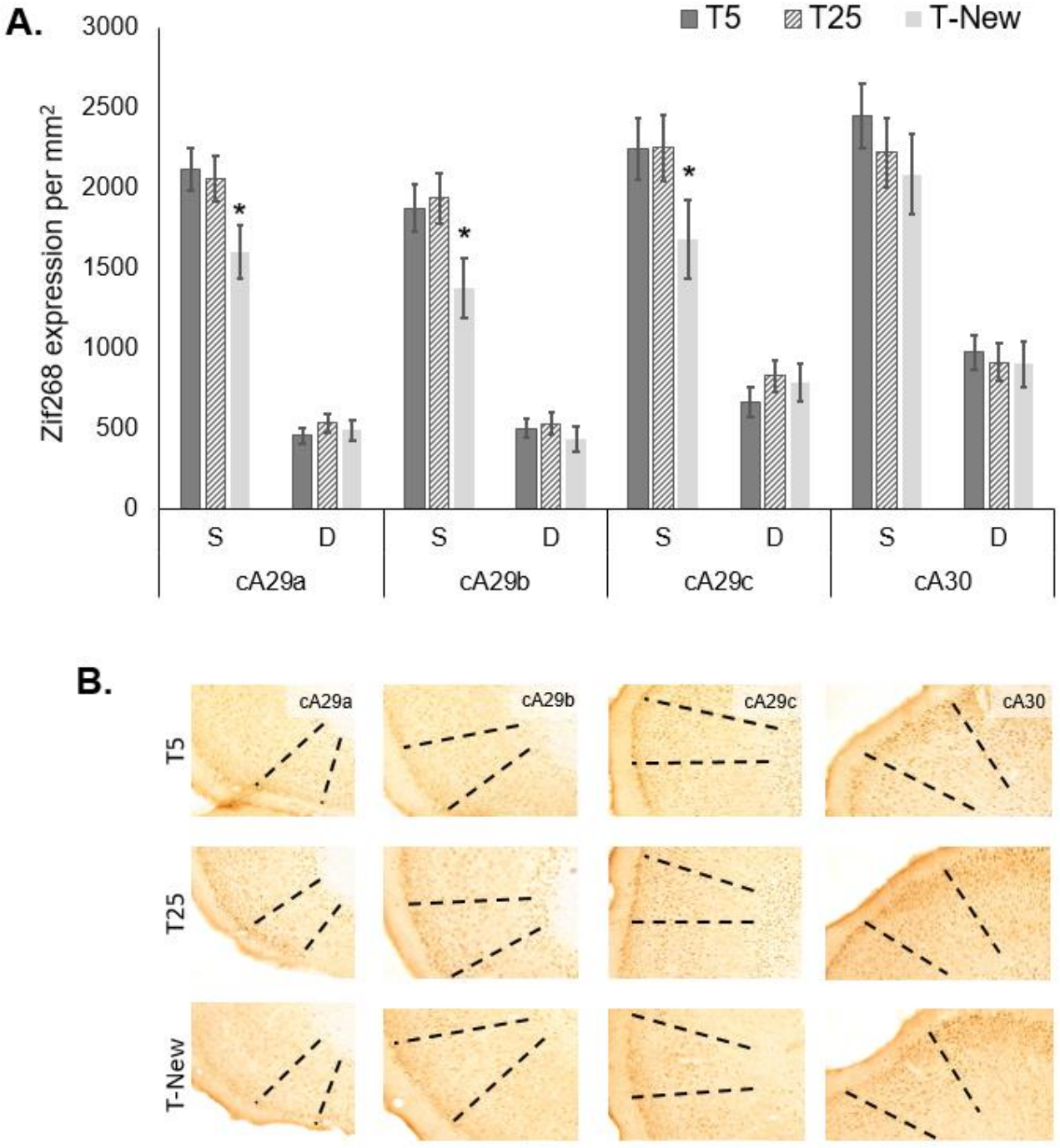

Figure 5.7. Zif268 expression in A29a, A29b, A29c and A30 of the caudal retrosplenial cortex (cA29 and cA30). **A.** cA29a-c and cA30 Zif268 expression/mm^2 in the three trace groups. **B.** 10x magnification photomicrograph examples Zif268 expression in the regions and layers of cA29a-c and cA30, decreased expression in T-New across the superficial layers of cA29 * $p<0.05$. Images represent the median in each group. D = deep layer; S = superficial layer; T5 = Trace Group, 5 day retention test after criterion (or maximum of 43 days); T25 = Trace Group, 25 day retention test after criterion (or maximum of 43 days); T-New = Trace Group, new association task at 25-days after acquisition of the original odour-trace-object task (or maximum of 43 days).

Perirhinal cortex

Zif268 expression in the perirhinal cortex is shown in Figure 5.8. Presentation of a novel odour/object pairing at day 25 (T-New) resulted in significantly lower Zif268 expression in the perirhinal cortex than in both T5 and T25 groups (Group main effect $F(2,19)=3.74$, $p=0.04$). The T-New Group differed from both T5 and T25 groups (T-New Group simple effect: versus T5, $F(1,19)=5.34$, $p=0.03$; and versus T25, $(F1,19)=6.37$, $p=0.02$). Zif268 expression in the two retention groups did not differ (T5 vs T25 $F(1,19)=0.05$, $p=0.82$).

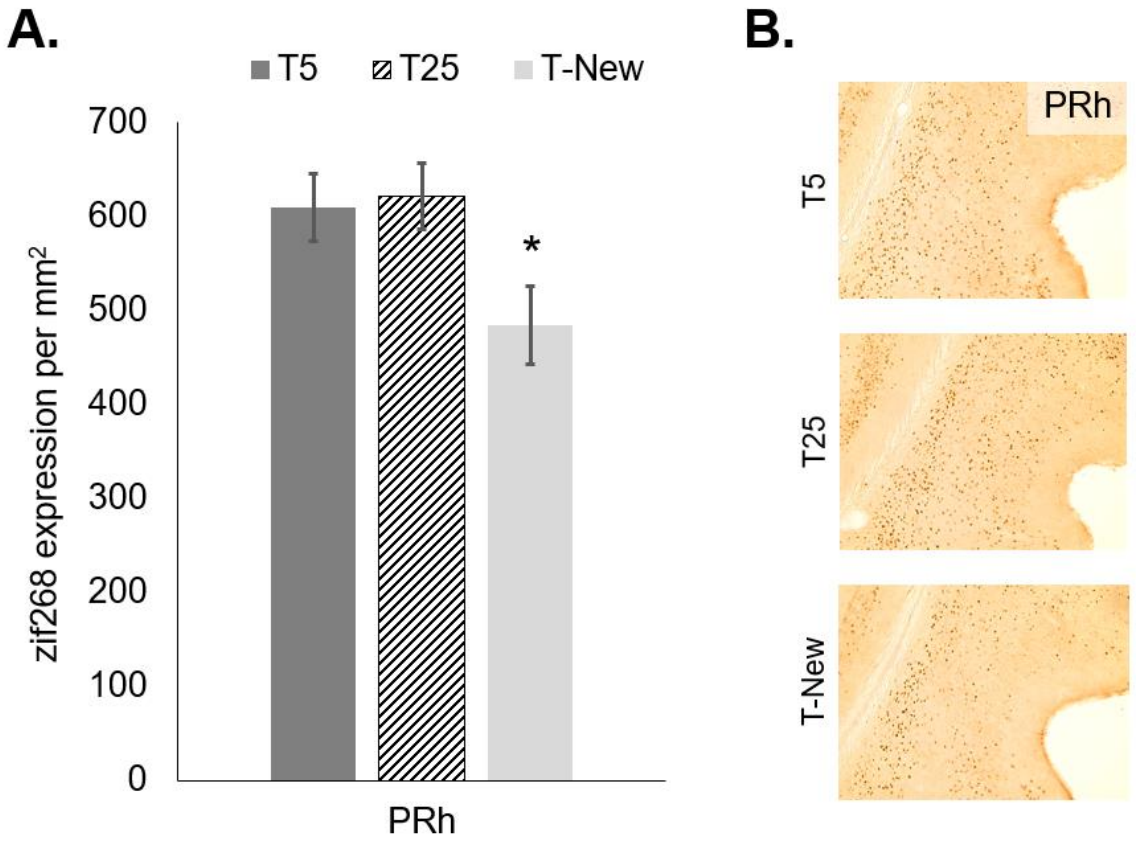

Figure 5.8. **A.** Zif268 expression per mm^2 in the perirhinal cortex *$p<0.05$. **B.** 10x magnification photomicrograph examples Zif268 expression in the perirhinal cortex. Decreased expression in group T-New, * $p<0.05$. Images represent the median in each group. PRh = perirhinal cortex; T5 = Trace Group, 5 day retention test after criterion (or maximum of 43 days); T25 = Trace Group, 25 day retention test after criterion (or maximum of 43 days); T-New = Trace Group, new association task at 25-days after acquisition of the original odour-trace-object task (or maximum of 43 days).

Entorhinal cortex

There were no significant differences in Zif268 expression between the three groups in the subregions of the entorhinal cortex (EC; Figure 5.9; Group ($F(2,13)=0.37$, $p=0.69$; Group by Subregion interaction $F(4,26)=0.16$, $p=0.95$).

Dorsal prefrontal cortex and cingulate cortex

There were no Group difference in Zif268 expression in the A32D region of the anterior cingulate cortex (Figure 5.9; Group main effect $F(2,19)=0.07$, $p=0.92$). Zif268 expression also did not differ between three groups in the A24a or A24b cingulate regions (Group main effect: A24a, $F(2,18)=2.29$, $p=0.12$; A24b, $F(2,19)=0.87$, $p=0.43$). Layer V in both cingulate regions expressed significantly lower counts of Zif268 than the two more superficial layers (II and III; Layer main effect: A24a, $F(2,36)=160.02$, $p<0.001$; A24b, $F(2,38)=76.97$, $p<0.001$).

Hippocampus and subiculum

Although there are significantly more Zif268 cells expressed in dorsal CA1 than any other subregion in the dorsal HPC (Figure 5.9; $F(3,54)=1687.97$, $p<0.001$), there was no Group ($F(2,18)=0.16$, $p=0.85$) or Group by Region interaction ($F(6,54)=1.34$, $p=0.25$). There was also significantly more Zif268 expression in the ventral CA1 than the ventral CA3 ($F(1,18)=288.67$, $p<0.001$), but again there was no Group ($F(2,18)=1.70$, $p=0.20$) or Subregion by Group interaction ($F(2,18)=2.24$, $p=0.13$). As with CA1 and CA3, the dorsal subiculum displayed higher Zif268 counts than the ventral subiculum ($F1,18)=24.65$, $p<0.001$) with no Group ($F(2,18)=1.99$, $p=0.16$) or Subregion by Group interaction ($F1,18)=1.43$, $p=0.26$).

Gustatory cortex (agranular and piriform cortices)

There was no group effect in either the agranular insular cortex (Figure 5.9; $F(2,18)=1.38$, $p=0.27$) or the piriform cortex (Group main effect $F(2,18)=0.77$, $p=0.47$; Layer x Group interaction, $F(2,18)=0.63$, $p=0.54$).

Parietal cortex

Similar levels of Zif268 were also found across groups in the parietal cortex (Figure 5.9; Group main effect $F(2,17)=2.09$, $p=0.15$; Layer x Group interaction, $F(6,51)=0.73$, $p=0.62$).

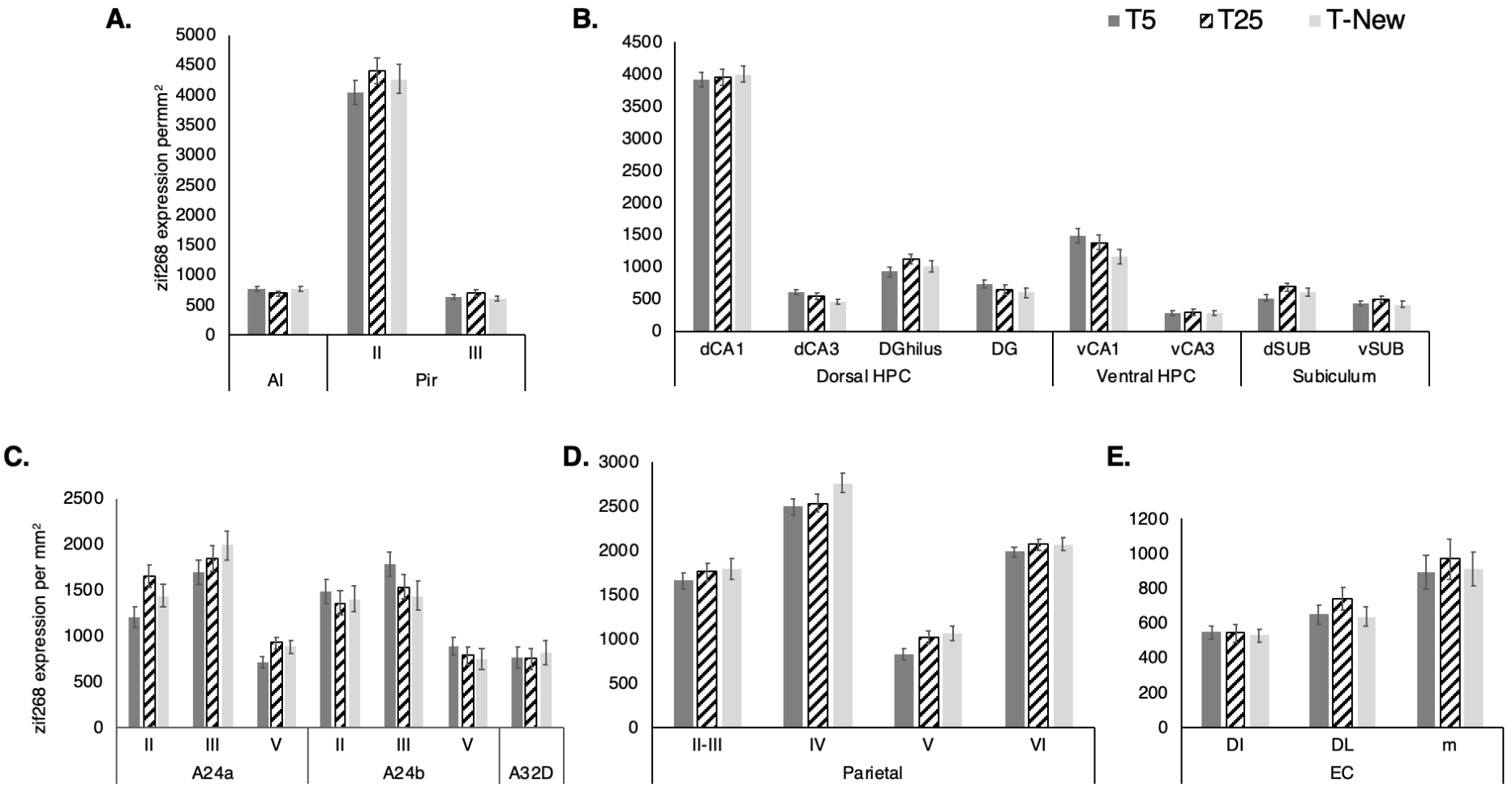

Figure 5.9. Zif268 expression in remaining regions of interest. **A.** Gustatory cortex Zif268 expression/mm2. **B.** Hippocampal Zif268 expression/mm2. **C.** Cingulate cortex Zif268 expression/mm2. **D.** Parietal cortex Zif268 expression/mm2. **E.** Entorhinal cortex Zif268 expression/mm2. Abbreviations: A24: A24 regions of anterior cingulate cortex; A24a: A24a region of anterior cingulate cortex; A24b: A24b region of anterior cingulate cortex; A32D: A32D region of anterior cingulate cortex; AI: agranular insular cortex; dCA1: Cornu Ammonis Area 1 of the dorsal hippocampus; dCA3: Cornu Ammonis Area 3 of the dorsal hippocampus; DG: dentate gyrus of the dorsal hippocampus; DGhilus: hilus of the dentate gyrus of the dorsal hippocampus; DI: dorsal intermediate entorhinal cortex; DL: dorsolateral entorhinal cortex; dSUB: dorsal subiculum; EC: entorhinal cortex; II: Layer II; III: Layer III; II-III: Layer II-III; IV: Layer IV; m: medial entorhinal cortex; Pir: piriform cortex; V: Layer V; VI: Layer VI; T5: Trace Group, 5 day retention test after criterion (or maximum of 43 days); T25: Trace Group, 25 day retention test after criterion (or maximum of 43 days); T-New: Trace Group, new association task at 25-days after acquisition of the original odour-trace-object task (or maximum of 43 days); vSub: ventral subiculum

5.5 Discussion

To identify the hippocampal and cortical regions involved in processing retention of the non-spatial odour-trace-object, we assessed the regional expression of the IEG Zif268 at 5- and 25-day recall. As mentioned above, Zif268 is rapidly regulated in response to behaviour (M.W. Jones et al., 2001), and can be used as an indication of neuronal activation following retention of a previously acquired memory or exposure to novel stimuli. In order to control for non-mnemonic IEG activation following the extended consolidation period, a third 'new learning' group were exposed to a novel pairing of stimuli 25-days post training on the original odour-trace-object procedure. The current study found increased activation in the superficial A32V prefrontal cortex following remote recall relative to recent recall or new learning of an odour-trace-object task. No clear effects of remote recall on this paired-associate task were found in other neural structures. Response accuracy was good in both retention groups demonstrating intact memory for the previously acquired paired-associate task, while the T-New group performed at chance. This pattern suggests that medial prefrontal cortex activation in Layer II was not simply related to the level of memory performance but may reflect consolidation of this non-spatial odour-trace-object task. Similarly, the delay between testing, per se, could also not explain the difference between the T25 and T5 groups. This evidence is consistent with previous work in which the recruitment of prefrontal cortical structures was reported for long-term retention of spatial memory in mice (Bontempi et al., 1999; Maviel et al., 2004). Despite examining both metabolic and IEG alterations in the prefrontal cortex, however, this previous work did not report the cell-layer distribution of activation in the prefrontal cortex.

In the current study, the superficial layer of the retrosplenial cortex exhibited upregulation of Zif268 in retention groups irrespective of recent or remote recall and new learning. This is contrary to an increase in retrosplenial Zif268 activation after recall of a

remote, but not recent, spatial memory (Maviel et al., 2004). Our findings suggests that the superficial retrosplenial, in a non-spatial task at least, is essential for recall in general rather than recent or remote retention for a non-spatial memory task.

The perirhinal cortex, despite being strongly implicated in novel object recognition (Brown & Aggleton, 2001), also showed Zif268 upregulation in the both retention groups compared to T-New. This interesting observation suggests that, although the perirhinal cortex is critically involved in object novelty, this region may also be involved in the recall of arbitrary associations for non-spatial information. There is debate concerning the perceptual-mnemonic dimension with respect to perirhinal cortex function (Bussey, Saksida, & Murray, 2005). Because recall activated more Zif268 expression than a novel odour-object pairing, the current findings provided clear evidence for a mnemonic representational role for the perirhinal cortex (Delhaye, Bahri, Salmon, & Bastin, 2019), at least for an odour-trace-object paired-associate memory.

Hippocampal activation showed no difference between recent and remote recall in the current experiment. This is in contrast to HPC downregulation in the consolidation of a spatial memory task. Kesner et al. (2005) have demonstrated that hippocampal CA1 recruitment is essential for temporal paired-associate acquisition in an object-trace-odour task. This is the first study to ask whether remote recall of a temporal context may differ from recent recall of that same temporal context. As we will see in Chapter 6, hippocampal CA1 activation is increased relative to an odour-object paired-associate task in the absence of the ‘trace’ component. Hence it is possible that CA1 activation may be obligatorily required for the odour-trace-object paired associated irrespective of consolidation. As the HPC is also involved in spatial memory, it would be interesting to compare Zif268 regulation following

recent and remote retention of various spatial and non-spatial temporal versions of paired-associate tasks.

There were limitations in the design of the present study. One such limitation is the lack of home cage controls to form baseline Zif268 activation levels in all regions of interest. This would provide a 'baseline' measure of non-specific neural activation unrelated to learning or exposure to salient stimuli. Such baseline activation would aid in the identification of regions critical to learning and memory. The current task appears to rely on the HPC to acquire the odour-object pairing across a temporal lag (Kesner et al., 2005). We cannot know baseline HPC activation levels even in the active controls (the new learning condition), as they are also exposed to the temporal component of the task. Home cage baseline Zif268 would also provide a control for perirhinal activation. This would help clarify whether 'new learning' Zif268 activation in the current task was higher than would be expected for when no novel stimuli are presented. One further limitation was that behavioural training was time-consuming (12 massed trials per rat, per day). This restricted the number of rats and paired controls being trained on the task.

Like most IEG studies, we assessed a single neural activation marker at only 90 minutes post retention testing, which may limit our understanding of the associations between behavioural and neural activation (Barry, Coogan, & Commins, 2016). It would be interesting to determine whether other activation markers would replicate the results we obtained. Adopting different markers of neural activity, such as different IEGs, combined with evidence from metabolic markers (i.e. cytochrome oxidase) would extend our findings. Frizzati et al. (2016) demonstrated that IEGs c-Fos and Zif268 do not produce identical results following MTT lesions. Although RSC regulation between the two markers was similar, hippocampal effects were more varied. This may be a reflection of activation markers responding

differently in particular brain regions and their sensitivity to different behavioural tasks (Barry et al., 2016).

In summary, this study provided evidence of activation within the extended memory system following recall of a temporal paired-associate non-spatial task. The findings identify an increase in cortical recruitment of the medial prefrontal A32V cortex only, that was specific to remote recall following acquisition of an odour-trace-object task. The next chapter provides a comparison of this odour-trace-object task with a non-temporal odour-object paired-associate task with respect to neural activation based on Zif268 expression.

Chapter 6.

A novel non-spatial paired-associate memory task: IEG correlates of the temporal context

6.1 Introduction

The previous chapter demonstrated that remote recall of an odour-trace-object paired associate task in intact rats, increased Zif268 expression in the superficial layer of medial prefrontal cortex. To expand on this, the current experiment examined the influence of this temporal 'trace' factor by comparing temporal and non-temporal versions of the odour-object task, described in Chapter 5.

The present study had two goals. The first was to assess acquisition rate of intact rats in both the odour-object and odour-trace-object paired-associate task. Previous comparison of acquisition of control rats on both object-odour (using a cheeseboard apparatus) and object-trace-odour (using a runway apparatus) paired-associate tasks suggested similar rates of task acquisition in intact rats (Gilbert & Kesner, 2002; Kesner et al., 2005). As already demonstrated in Chapter 5, intact rats were able to acquire and recall (recent and remotely) the non-spatial temporal odour-trace-object paired-associate task. A direct within-task comparison of trace versus no trace is preferable, especially for future research that examines their neurobiological correlates. This was done by having one variation that does not include a trace and one that explicitly includes a 10 second trace between the object and the odour. Based on work from Kesner, it was expected that both trace and no-trace conditions of the paired associate tasks would be acquired at the same rate.

The second, and primary, goal was to identify the pattern of neural activation associated with recall of both trace and no-trace variations of the runway task. We anticipated finding different levels of IEG activation within the HPC, and specifically dorsal CA1, based on lesion evidence from Kesner et al. (2005). They reported that dorsal CA1 lesions removed the ability to acquire a temporal paired-associate task in rats, but not a non-temporal object-odour task, albeit the latter was tested in a different apparatus and procedure. To ensure that recall of these non-spatial paired-associate tasks was reflected by Zif268 levels, all rats in the current study receive the post-acquisition retention test 5 days after training. This meant that IEG activation reflected temporal or non-temporal recall rather than differences that might emerge from a long-term retention test. The same regions as in the previous chapter were examined here, to learn whether regions beyond dorsal CA1 also show changes associated with the trace aspect of the paired-associate task. No predictions were made regarding other regions, beyond the prospect that dorsal CA3 should show no difference, given CA3 lesions did not impair acquisition of even the trace version of the task in Kesner's study (2005).

6.2 Materials and Method

6.2.1 Subjects

Eighteen 12-month old male Long-Evans rats were used. They were bred and housed in the University of Canterbury's animal facility. Eight rats (Group T5) provided data for both the current No-Trace versus Trace comparison of IEG affects as well as the memory consolidation study. Housing conditions and food restriction procedures were the same as outlined in Chapter 5. All procedures carried out were approved by the Animal Ethics Committee of the University of Canterbury (#2017/20R).

6.2.2 Simple discrimination and paired-associate tasks

Apparatus

The apparatus and general procedures were the same as described in Chapter 5. The difference was that one group (No Trace) was trained without a delay between the presented odour and subsequent object. For the acquisition on the No Trace task, rats started in compartment B (A, Figure 6.1).

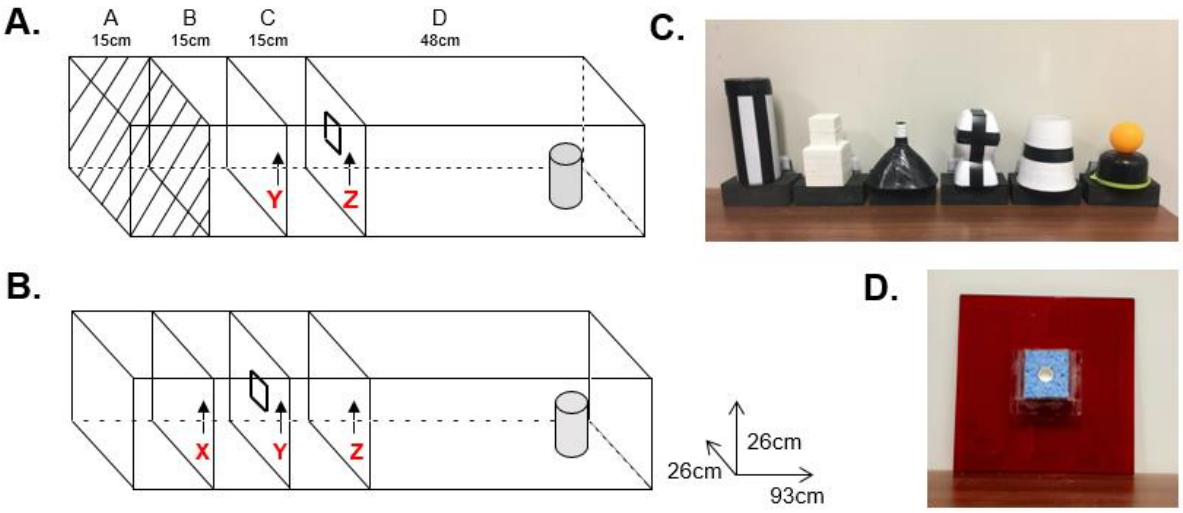

Figure 6.1. Schematic of the runway used to acquire the simple odour discrimination task, the simple object discrimination task, and the two odour-object association memory tasks (trace and no-trace). **A.** Placement of doors for the **odour-object paired-associate task (no trace)**. Compartment B was used as the start box and the odour was presented on Door Z (end of Compartment C). The far end of Compartment D presented the moveable object mounted by a hinge above the food receptacle. **B.** Placement of doors for the **odour-trace-object task.** Compartment A was used as the start box and the odour receptacle now on Door Y was presented at the end of Compartment B. Compartment C provided the trace/delay compartment. **C.** Objects used in the simple (left) and paired associate (right) tasks. **D.** Odour receptacle and door used in the simple and paired associate tasks. For simple odour or simple object discrimination tasks, see Figure 5.1 legend (Chapter 5).

Odour/object pre-surgery shaping

Habituation and shaping procedures were the same as in Chapter 5.

Simple odour and object discrimination tasks

Simple discrimination tasks were carried out as defined in Chapter 5. Rats were trained on the simple odour and simple object discrimination task until they reached criterion (80% correct over 2 consecutive days).

Trace and no-trace odour-object paired-associate memory tasks

Rats were randomly assigned to the No Trace (NT5; n=10) and Trace (T5; n=8) conditions. Rats in the Trace condition were trained on the odour-trace-object paired-associate task in the same manner as Chapter 5. The difference in this study being that rats in the No Trace condition began training in compartment B (**B**, Figure 6.1) and were not subjected to the 10 second trace between stimuli. Latency to interact with the object was measured from the time door Z was lifted in both No Trace and Trace conditions, thus providing an equal distance before the rat was able to approach the object at the end of compartment D. All rats were trained on the same 'go, no-go' reward structure as rats in Chapter 5.

Post-acquisition recall test

Recall of the paired-associate task (No Trace or Trace) was conducted 5 days either after the rat reached criterion or at the end of 43 days of formal training. This recall session used 12 massed trials in identical fashion and with the same odours and objects as used in training.

6.2.3 Histological procedures

Perfusion and tissue preparation

The Zif268 induction procedure was carried out as defined in Chapter 5. Perfusion procedure and collection of coronal brain sections were as outlined in Chapter 5.

Zif268 immunohistochemistry

Zif268 staining and image processing was completed in the same manner as Chapter 5.

6.2.4 Regions of interest

Regions of interest (ROIs) were selected consistent with Chapter 5. These regions included hippocampal and cortical regions anticipated as relevant for the No Trace versus Trace comparison. To facilitate reference Figure 5.2 has been replicated here as Figure 6.2.

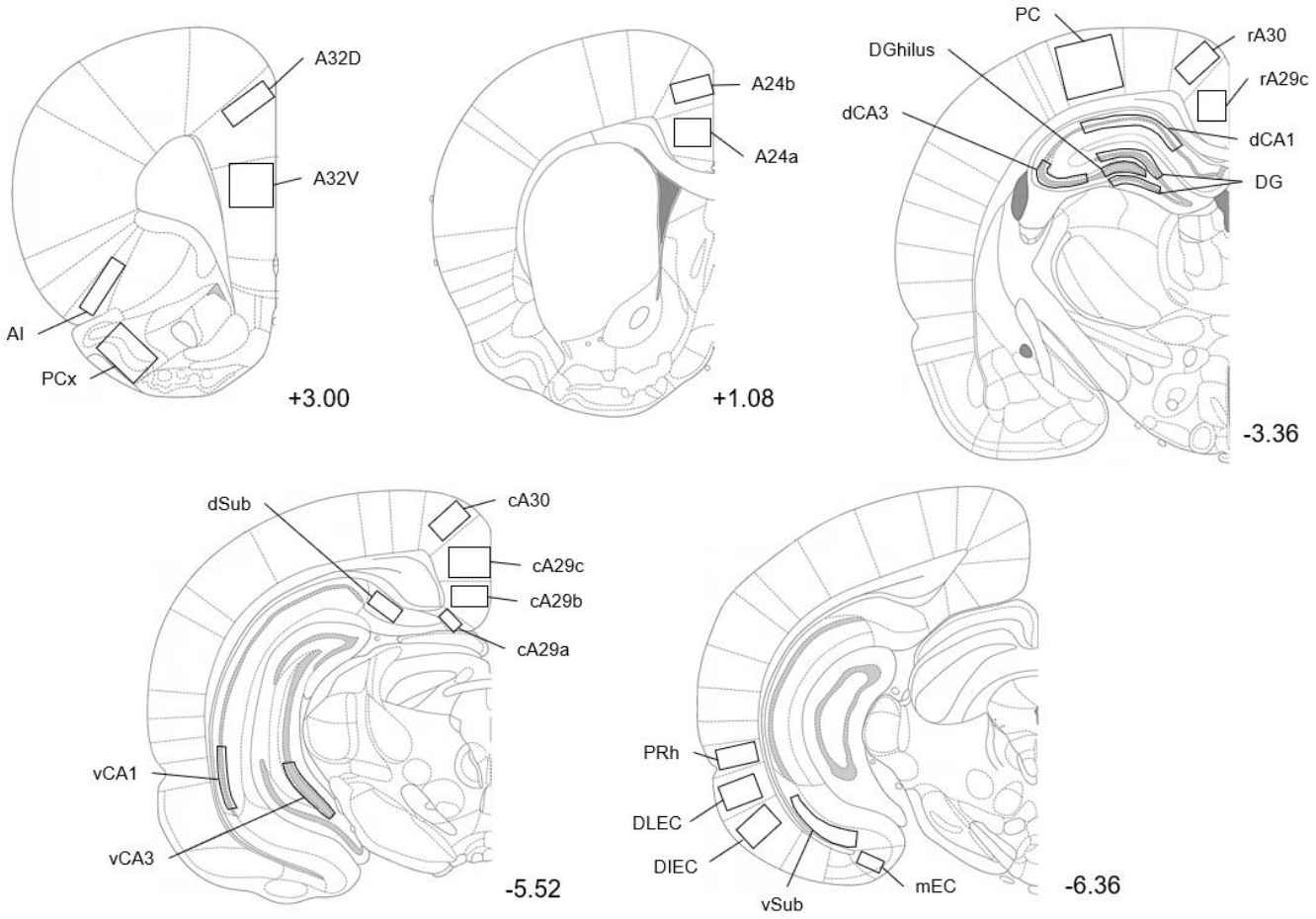

Figure 6.2. Regions of interest included in quantification of Zif268 expression. This replicates Figure 5.2, Chapter 5. A24a/b = area 24a/b of the anterior cingulate cortex; A32D/V = dorsal/ventral area 32 of the anterior cingulate/prefrontal cortex; AI = agranular insular cortex; dCA1 = Cornu Ammonis Area 1 of the dorsal hippocampus; dCA3 = Cornu Ammonis Area 3 of the dorsal hippocampus; DG= dentate gyrus of the dorsal hippocampus; DGhilus = hilus of the dentate gyrus of the dorsal hippocampus; DIEC = dorsal intermediate entorhinal cortex; DLEC = dorsolateral entorhinal cortex; dSub = dorsal subiculum; mEC = medial entorhinal cortex; PC = piriform cortex; PRh = perirhinal cortex; r/cA29a/b/c = rostral/caudal area 29a/b/c of the granular retrosplenial cortex; r/cA30 = rostral/caudal area 30 of the retrosplenial cortex; vCA1 = Cornu Ammonis Area 1 of the ventral hippocampus; vCA3 = Cornu Ammonis Area 3 of the ventral hippocampus; vSub = ventral subiculum.

6.2.5 Statistical analysis.

Statistical analyses were conducted using Statistica (v13; Dell Inc.) in the same manner as Chapter 5.

6.3 Results

6.3.1 Simple discrimination

Acquisition of both the simple odour and simple object discrimination is shown in Figure 6.3. There were no Trace (T5) vs No Trace (NT5) group differences in the acquisition for the simple odour discrimination (Group main effect, $F(1,16)=0.29$, $p=0.59$; Day x Group interaction, $F(6,96)=0.71$, $p=0.64$). Both groups rapidly acquired the simple object discrimination, although Group T5 improved faster reflected by better performance on day 2 (Group main effect, $F(1,16)=0.04$, $p=0.84$; Day x Group interaction, $F(6,96)=2.51$, $p=0.02$); Day 2 simple main effect, $F(1,16)=4.759$, $p=0.044$). Both T5 and NT5 groups tool similar days to reach criterion for each simple discrimination (Odour: NT5 M=5.20, SD=0.91; T5 M=4.87, SD=1.12, $t=0.67$, $p=0.50$; Object: NT5 M=6.30, SD=0.94; T5 M=6.37, SD=0.91, $t=-0.16$, $p=0.86$).

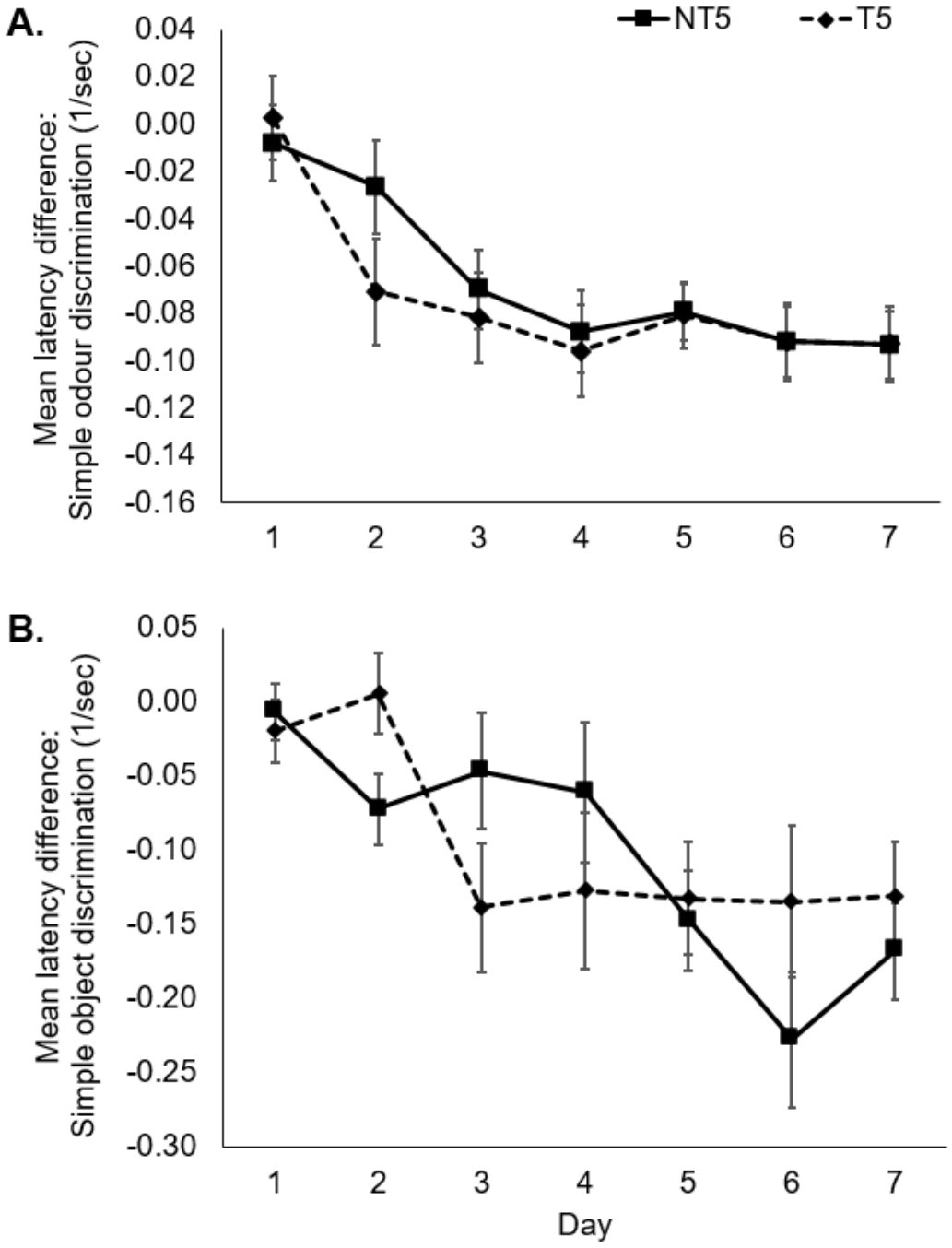

Figure 6.3. **A.** Simple odour and **B.** simple object discrimination task acquisition (reciprocal mean latency difference). 0.00 represents no difference in responding and -0.10 corresponds to approximately 3 second latency difference between 'go' and 'no-go' trials. NT5= No-Trace Group, 5-day retention test; MLD = mean latency difference; T5 = Trace Group, 5-day retention test.

6.3.2 No-Trace versus Trace odour-object association memory and retention

Task acquisition of the reciprocal mean latency difference is show in Figure 6.4. Both NT5 and T5 groups acquired the task at the same rate (sessions to criterion t-test; NT5 M=37.10,

SD=5.50; T5 M=36.75, SD=5.84, t=-0.13, p=0.89). The first rat reached criterion at day 29. Of the 18 rats, 12 rats acquired the object-odour association with 3 rats in each condition not reaching criterion. The final mean latency difference score was carried forward for those rats that had reached criterion prior to day 43. Both groups NT5 and T5 had a similar rate of acquisition, although Group T5 showed faster acquisition at initial training stages (Group main effect, $F(1,16)=3.86$, $p=0.06$; Block x Group, $F(10,160)=1.87$, $p=0.05$). By the final block of trials (Block 11), however, there was no difference in performance between the groups (NT5 M=-0.45, SD=0.09; T5 M=-0.49, SD=0.05, t=-0.89, p=0.38).

Both groups performed similarly in the retention test, and despite a small mean drop in performance there was no significant difference relative to the last block of training (Group main effect $F(1,16)=0.65$, $p=0.431$; Block x Group interaction $F(1,16)=0.01$, $p=0.919$). The No-Trace and Trace conditions did not differ in retention test performance when tested 5-days after acquisition (NT5: M=-0.44, SD=0.12 and NT5: M=-0.47, SD=0.099, t=-0.56, p=0.577).

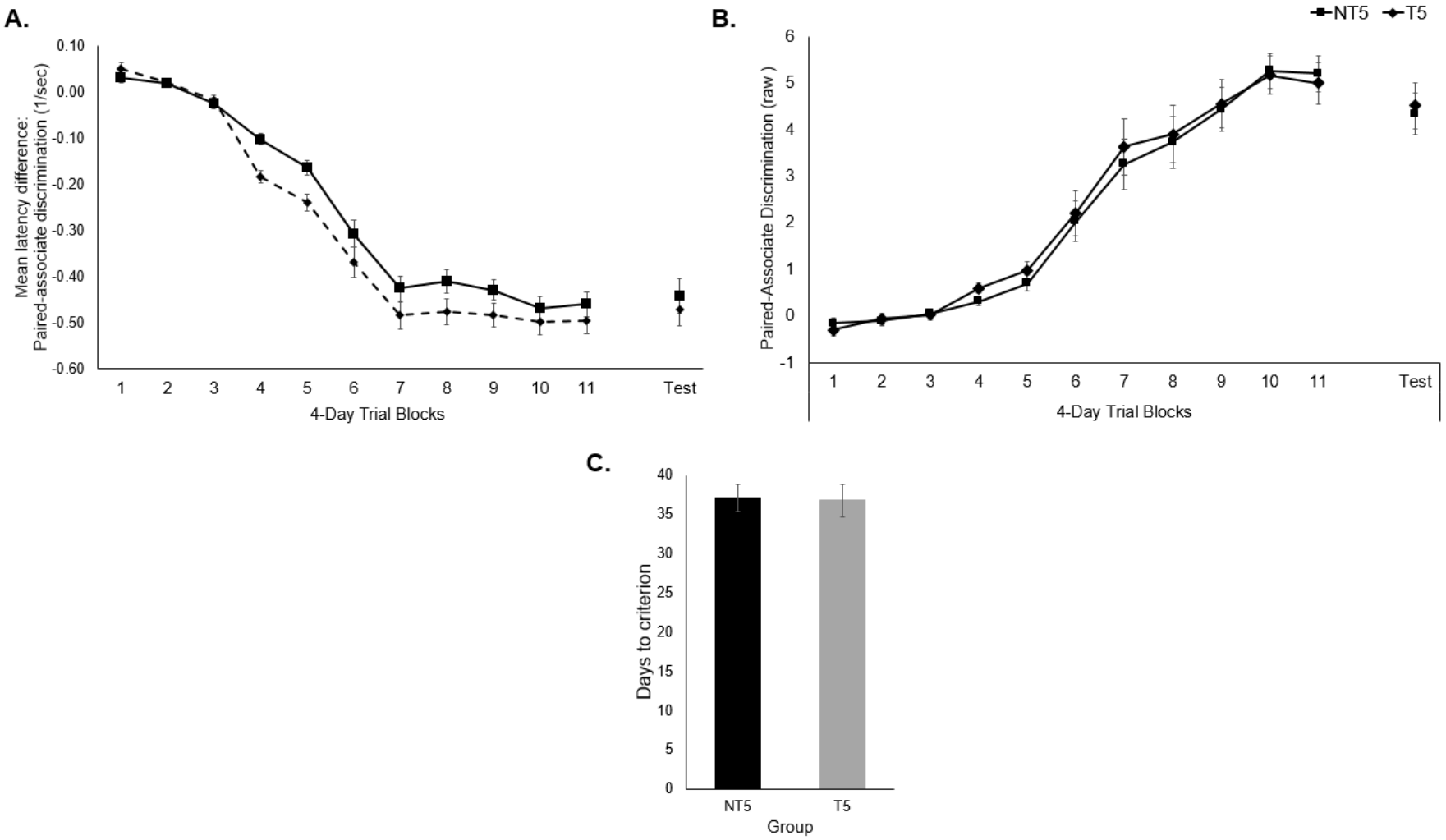

Figure 6.4 Acquisition and retention of the odour-object and odour-trace-object paired-associate memory task in 4-day trial blocks. **A.** Normalised acquisition latency scores; mean latency difference of 0.00 represents no difference, and -0.50 represents approximately a 5 second difference in responding to the 'go' and 'no-go' trials. **B.** Raw mean latency difference discrimination scores in seconds. **C.** Average days to criterion for each group. Error bars = standard error. NT5 = No Trace Group, 5 day retention test after criterion (or maximum of 43 sessions); T5 = Trace Group, 5 day retention test after criterion (or maximum of 43 days).

6.3.3 Zif268 IEG analysis

Zif268 counts were analysed across the ROIs comparing Groups NT5 and T5 following the retention test. Significant group differences were limited to the hippocampus and dysgranular (A30) retrosplenial cortex.

Hippocampal Zif268 expression

The expression of Zif268 in hippocampus after the retention test 5-days after acquisition is shown in Figure 6.5. Zif268 expression in the dorsal hippocampal subfields was substantially higher in CA1 than in other subfields and lowest in CA3 (Subregion, $F(3,45)=1111.79$, $p<0.01$). There was a Group main effect ($F(1,15)=7.08$, $p=0.01$), with higher expression in the Group T5 in both dorsal CA1 and CA3 (Group by Subregion interaction $F(3,45)=3.74$, $p=0.01$; simple main effect of Group for CA1, $F(1,15)=6.54$, $p=0.02$, and for CA3, $F(1,15)=10.53$, $p<0.01$). Compared to the dorsal hippocampus, Zif268 expression was much reduced in ventral CA1 and CA3, although still considerably higher in CA1 ($F(1,16)=196.29$, $p<0.01$). For the ventral hippocampus, there was no Group ($F(1,16)=0.05$, $p=0.81$) or Group by Subregion interaction ($F(1,16)<0.01$, $p=0.95$). There were also no Group ($F(1,15)<0.01$, $p=0.98$) or Group by Subregion ($F(1,15)=0.38$, $p=0.54$) effects for the subiculum.

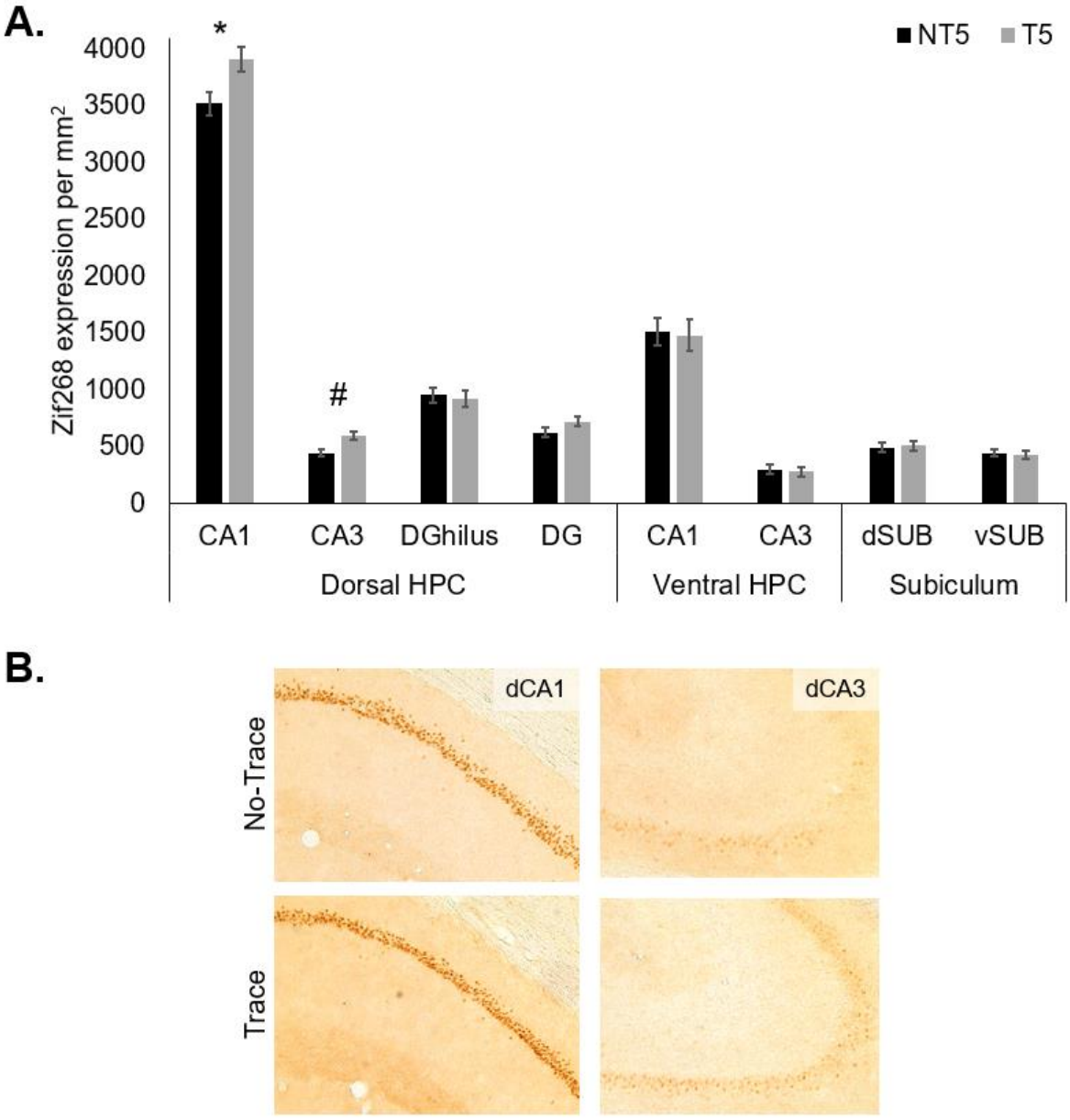

Figure 6.5 Zif268 expression in the hippocampal formation following the 5-day retention test. **A.** Hippocampal and subicular Zif268 expression/mm2. *p<0.05, #p<0.01 **B.** 10x magnification photomicrograph examples of Zif268 expression in the dorsal CA1 and CA3. Images represent the median for each group. CA1 = Cornu Ammonis Area 1 of the Hippocampus; CA3 = Cornu Ammonis Area 3 of the Hippocampus, DG = dentate gyrus of the dorsal hippocampus; DGhilus = hilus of the dentate gyrus of the dorsal hippocampus; NT5 = No Trace Group, 5 day retention test after criterion (or maximum of 43 sessions); T5 = Trace Group, 5 day retention test after criterion (or maximum of 43 sessions).

Retrosplenial cortex Zif268 expression

Retention of the trace paired-associate task resulted in higher expression in the rostral retrosplenial cortex (**A**. Figure 6.6). However, only the dysgranular A30 region reached

significance in this group comparison (A29c Group main effect $F(1,14)=3.42$, $p=0.08$; A30 $F(1,15)=4.89$, $p=0.04$). Group T5 expressed higher Zif268 in both layers of A30, but this was to a greater extent in the superficial layer (Layer x Group interaction $F(1,15)=6.97$, $p=0.01$; simple main effect Superficial $F(1,15)=6.77$, $p=0.01$; Deep $F(1,15)=1.54$, $p=0.23$). There was no Layer by Group interaction in A29c ($F(1,14)=0.10$, $p=0.75$).

Expression of Zif268 in caudal A29 and A30 regions of the RSC following the 5-day retention test is shown in **C**. Figure 6.6. There were no group differences seen across the three caudal A29 regions (main Group effect: A29a $F(1,14)=0.87$, $p=0.36$; A29b $F(1,14)=3.16$, $p=0.09$; A29c $F(1,14)<1$, $p=0.96$). Although Group T5 expressed higher counts in the superficial layer across the three A29 regions there were no Layer x Group interactions (A29a $F(1,14)=1.41$, $p=0.25$; A29b $F(1,14)=0.56$, $p=0.46$; A29c $F(1,14)=2.68$, $p=0.12$). In contrast, Group NT5 expressed higher counts of Zif268 in both the superficial and deep layers of A30. However, this group difference did not reach significance ($F(1,14)=3.39$, $p=0.08$) and there was no Layer by Group interaction ($F(1,14)=0.26$, $p=0.61$).

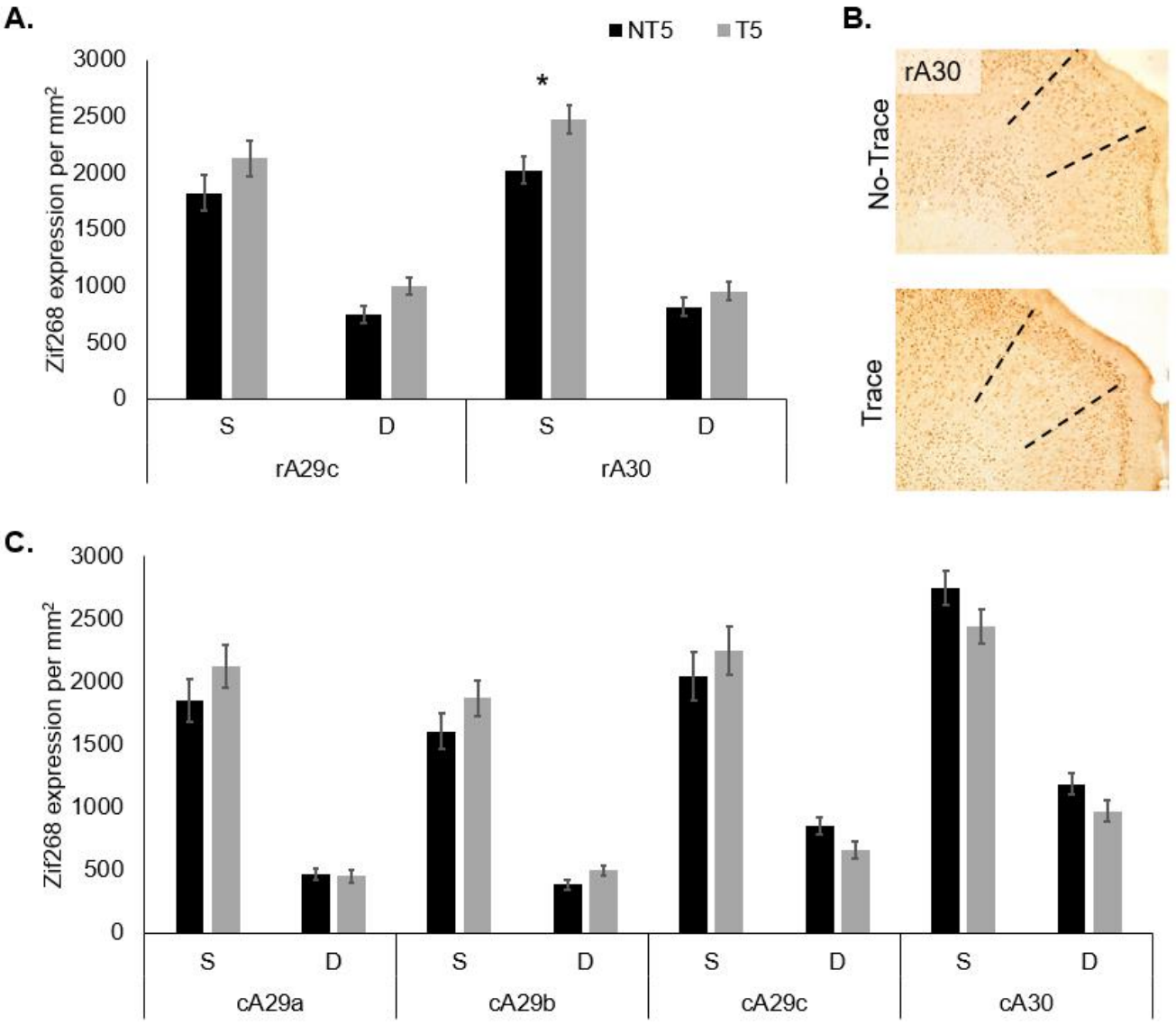

Figure 6.6 Zif268 expression per mm^2 in the retrosplenial cortex (Mean ± Std Err). **A.** Rostral A29c and A30 Zif268 expression per mm2 in Groups NT5 and T5. **B.** 10x magnification photomicrographs of the rostral layers of A30. Images represent the median for each group. **C.** Zif268 expression per mm^2 in caudal A29 and A30 retrosplenial cortex cA29a = caudal area 29a of the retrosplenial cortex; cA29b = caudal area 29b of the retrosplenial cortex; cA29c = caudal area 29c of the retrosplenial cortex; cA30 = caudal area 30 of the retrosplenial cortex; D = deep layer; NT5 = No Trace Group, 5 day retention test after criterion (or maximum of 43 sessions); rA29c = rostral area 29c of the retrosplenial cortex; rA30 = rostral area 30 of the retrosplenial cortex; S = superficial layer; T5 = Trace Group, 5 day retention test after criterion (or maximum of 43 sessions)

Prefrontal and cingulate Zif268 expression

Expression of Zif268 in the prefrontal (A32V) and anterior cingulate cortices (A32D, A24a/b) can be seen in **A**, Figure 6.7. No group differences in Zif268 expression were evident in the

ventro-medial prefrontal cortex (A32V; Group main effect, F(1,16)=0.02, p=0.88; Layer by Group effect, F(3,28)=0.34, p=0.79). Both Groups NT5 and T5 presented the same levels of Zif268 in the dorso-medial prefrontal cortex region A32D (Group main effect, F(1,16)=0.01, p=0.91). No-Trace or Trace conditions did not alter Zif268 expression in the A24 anterior cingulate regions (Group main effect A24a F(1,16)=0.03, p=0.85; A24b F(1,16)=2.92, p=0.10). There was also no Layer by Group effect in the A24 regions (A24a F(2,32)=0.21, p=0.80; A24b F(2,32)=0.53, p=0.59).

Gustatory cortex

Zif268 expression in the agranular insular and piriform cortices in the gustatory cortex and the parietal cortex is shown in **B**, Figure 6.7. There was no group difference in Zif268 expression in the agranular insular cortex (NT5 M=722.540, SD=111.099: T5 M=771.670 SD=76.520; t=1.047, p=0.311). Similarly, there was no significant difference in expression in the piriform cortex (Group main effect, F(1,15)=3.186, p=0.094; Layer x Group interaction, F(1,15)=3.545, p=0.079).

Parietal cortex

Groups NT5 and T5 expressed similar levels of Zif268 in the layers of the lateral parietal association cortex (**C**, Figure 6.7; Group main effect, F(1,15)=0.117, p=0.737; Layer by Group interaction, F(3,45)=1.62, p=0.334).

Parahippocampal cortex

Zif268 expression of the parahippocampal regions (perirhinal and entorhinal cortices) for Groups NT5 and T5 are shown in **D**, Figure 6.7. Both Groups NT5 and T5 expressed similar levels of Zif268 in the perirhinal cortex (NT5 M=591.886 SD=109.895, T5 M=609.022 SD=114.495, t=0.314, p=0.757). Zif268 expression did not significantly differ between the

two groups in the entorhinal cortex ($F(1,10)=3.417$, $p=0.094$). While there appears to be more Zif268 expressed by Group NT5 across the subregions on the entorhinal cortex, especially in the medial subregion, this pattern did not reach significance (Subregion x Group $F(2,20)=0.726$, $p=0.495$).

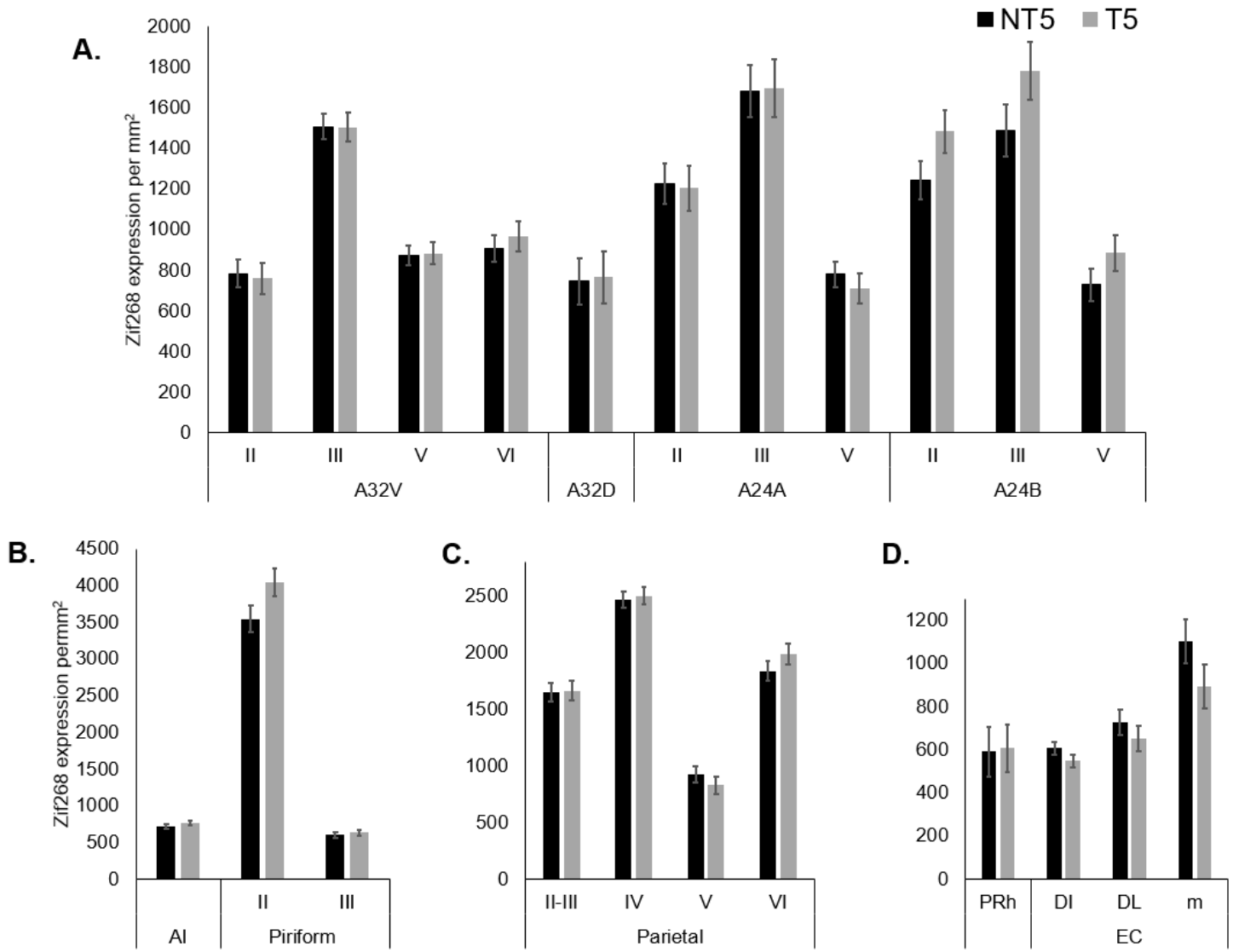

Figure 6.7. Zif268 expression in remaining regions of interest. **A.** Anterior cingulate cortex Zif268 expression/mm2. **B.** Gustatory Zif268 expression/mm2. **C.** Parietal cortex Zif268 expression/mm2. **E.** Parahippocampal cortex Zif268 expression/mm2 (Mean ± Std.Err). Abbreviations: A24a: A24a region of anterior cingulate cortex; A24b: A24b region of anterior cingulate cortex; A32D: A32D region of anterior cingulate cortex; A32V: A32V region of prefrontal/anterior cingulate cortex; AI: agranular insular cortex; DI: dorsal intermediate entorhinal cortex; DL: dorsolateral entorhinal cortex; EC: entorhinal cortex; II: Layer II; III: Layer III; II-III: Layer II-III; IV: Layer IV; m: medial entorhinal cortex; NT5: No-Trace Group, 5 day retention test after criterion (or maximum of 43 days); PRh: perirhinal cortex; V: Layer V; VI: Layer VI; T5: Trace Group, 5 day retention test after criterion (or maximum of 43 days).

6.4 Discussion

We assessed the regional expression of Zif268 5-days post task acquisition to identify neural activation specific to recall of an odour-object (without trace) and an odour-trace-object paired associate task. The examination of neural activation related to these tasks in intact rats that acquired the task provides evidence of task specific activation. As predicted, intact rats were able to acquire the non-temporal at a similar rate to criterion to rats trained on the odour-trace-object task, although rats in the trace condition appeared to learn the discrimination at a faster rate than rats in the no-trace condition. By the end of training, all rats showed similar levels of performance. This is the first study, to our knowledge, that examines neural activation in intact rats on recall of both No Trace and Trace non-spatial paired-associate tasks. The benefit of examining neural activation is that it describes a potential positive, active role for individual neurons, including subregional/layer-specific neurons, in a given task. Lesion effects, by contrast, may reflect secondary changes occurring in other structures as a consequence of the injury.

The findings from this study provide clear evidence of hippocampal recruitment on the odour-trace-object paired associate task. As predicted, dorsal CA1 neurons showed increased Zif268 expression on the 5-day retention test of the Trace task compared to the No Trace condition. This is consistent with the study by Kesner et al. (2005) where CA1 lesion rats were unable to acquire a similar task. This suggests that forming an arbitrary association across a temporal lag requires the recruitment of CA1. The increase of Zif268 expression found in the dorsal CA3, however, was not expected. Rats with dorsal CA3 lesions were able to acquire the object-trace-odour task (Kesner et al., 2005). This finding could suggest that, although acquisition was spared following CA3 lesions, these neurons are recruited in a normal intact hippocampus when tested on the trace paired-associate task. Both of these

findings will be re-assessed in the replication using intact sham-lesion rats in the next chapter, which looks at the effects of ATN lesions on these tasks.

Increased Zif268 activation in the superficial layer of the rostral dysgranular retrosplenial cortex (A30) was also observed following recall of the odour-trace-object paired-associate task. This pattern of activation was also seen through the granular retrosplenial cortex but was not significant. Despite being commonly implicated in spatial learning and memory (Aggleton & Pearce, 2001; Wyss & Van Groen, 1992), this activation implies that the retrosplenial cortex may also be recruited in this non-spatial temporal paired-associate task. The dense connections between the HPC and retrosplenial cortex may account for the increase in activation following recall on the trace task, a seemingly hippocampal-dependent paired-associate task (van Groen & Wyss, 1990b, 1992, 2003).

Limitations and future directions for the current study are similar to those outlined in Chapter 5. The greatest limitation to this chapter was the lack of control animals, although this does not negate the specific between task differences. Unlike Chapter 5, no active controls (new learning group) were tested to determine whether neural activation measure was due to learning and recall, or a by-product of being put back into a familiar apparatus. The latter, however, could be expected to be similar across the two groups because their performance on the 5-day tests was similar. ‘Baseline’ measures of non-specific neural activation (caged controls) would, however, provide valuable information regarding activation levels in the hippocampus, for example, relative to rats not exposed to any task-relevant stimuli, temporal or non-temporal.

Animal lesion studies have proven that hippocampal and diencephalic structures are critical for spatial memory function. Evidence shows both the HPC and ATN are critical for

acquisition in spatial variants of paired-associate memory tasks (object-place or odour-place) (Gibb et al., 2006; Gilbert & Kesner, 2002, 2003; Kesner et al., 2002; Kesner et al., 2005; Sziklas & Petrides, 1999). The impairments in these tasks, however, may be explained by the severe impairment in the spatial processing aspect of the task, rather than the inability to form an arbitrary association. For lesions that affect the hippocampal system and related neural structures, it is therefore beneficial for us to discover neural activation associated with non-spatial temporal paired-associate tasks. Using evidence from both the current study and Chapter 5, it was predicted that ATN lesions produce deficits in the odour-trace-object task specifically (Chapter 7), providing a potential option (odour-object) to further examine neurobiological correlates of long-term consolidation.

Chapter 7.
No-Trace/Trace Odour-Object Association and ATN Lesions

7.1 Introduction

The anterior thalamic nuclei (ATN) are part of a distributed network associated with episodic memory, as discussed in Chapters 3 and 4. Injury to or dysfunction of the ATN is evident in clinical patients with diencephalic injury who present with an amnesic syndrome, such as Korsakoff's syndrome and thalamic stroke causing MTT lesions (Carlesimo et al., 2011; Harding et al., 2000; Kopelman, 2015). Memory deficits following ATN lesions in rats are often comparable to the pattern of behavioural deficits following HPC lesions (Dumont & Aggleton, 2013; Mitchell & Dalrymple-Alford, 2006; Sziklas & Petrides, 2007; Wolff et al., 2006). Along with the extensive neural connections between the ATN cingulate/retrosplenial cortex, prefrontal cortex and hippocampal subiculum, these memory deficits reinforce the idea that the ATN are a key node in the 'hippocampal-diencephalic-cingulate' memory network (Bubb et al., 2017). The interdependence of the ATN and hippocampal formation is supported by evidence from cross-lesion studies, described briefly in Chapter 4. That is, contralateral lesions to the ATN and dorsal HPC produced spatial memory deficits, while ipsilateral lesions did not (Henry et al., 2004; Warburton et al., 2001). In addition, lesions to the ATN consistently cause IEG hypoactivation in the RSC (Dumont et al., 2012; Perry et al., 2018; Poirier et al., 2008). This body of evidence supports the ATN's involvement in an extended circuit of brain regions that function together to support memory, and in particular episodic memory.

There are no direct connections between the ATN and hippocampus proper (Bubb et al., 2017). This may explain why the impact of injury to the ATN on neural activation of the HPC has been inconsistent (Dumont et al., 2012; Dupire et al., 2013; Jenkins, Dias, Amin, & Aggleton, 2002; Jenkins, Dias, Amin, Brown, et al., 2002; Loukavenko, Ottley, Moran, Wolff, & Dalrymple-Alford, 2007). Nonetheless, ATN lesions reduce the microstructural integrity of CA1 neurons and are associated with impaired spatial memory performance (Harland et al., 2014). Further, reduced spine integrity in CA1 neurons has also been reported after MTT lesions, which would exert their influence through dysfunction of the ATN (Dillingham et al., 2019). ATN lesions, however, do not seem to impact spatial CA1 units (Frost et al., 2020). CA1 neurons provide one of the primary sources of HPC outputs (Aggleton, 2012), so the functional impact of ATN injury on memory function remains unclear. One possibility is that ATN lesions produce deficits on non-spatial tasks that are sensitive to HPC lesions, and specifically CA1 injury.

Impaired acquisition of paired-associate tasks after HPC injury is generally found when individual stimuli are presented across spatial or temporal contexts. For example, hippocampal and selective CA3 injury disrupts acquisition of a spatial paired-associate task (Gilbert & Kesner, 2002, 2003). A similar deficit has been reported after ATN lesions (Gibb et al., 2006). As described in Chapter 4, acquisition of non-spatial object-odour paired associate tasks were not dependent on the HPC. Kesner et al. (2005) adapted the non-spatial object-odour paired-associate task by introducing a temporal component to the task. CA1 lesions, but not the CA3 lesions, prevented acquisition of this object-trace-odour task, that is, a temporal non-spatial paired-associate task. There is some evidence that ATN lesions impair behaviour beyond spatial memory when the task depends on a temporal component. ATN lesions abolished memory for the temporal order of sequentially-presented odours, and impaired temporal memory for objects, without affecting not simple discriminations of odours

or objects (Dumont & Aggleton, 2013; Wolff et al., 2006). These ATN lesion findings match deficits following HPC lesions in temporal order tasks (Fortin et al., 2002; Kesner et al., 2002).

In previous paired-associate tasks, ATN involvement appears to be limited to those in which one component was spatial. For example, Dumont et al. (2014) tested the effects of ATN lesions in a range of biconditional discrimination tasks. Such tasks require discrimination between correct and incorrect pairings of stimuli in both spatial and non-spatial contexts. They found that ATN lesions produced selective and severe deficits only in tasks requiring rats to use distal spatial cues. Consistent with their findings, earlier work reported that ATN lesions impaired odour-place and object-place paired-associate tasks (Gibb et al., 2006; Sziklas & Petrides, 1999). The results reflect similar findings with HPC lesions (Jo & Lee, 2010; Sziklas & Petrides, 2002). These studies were discussed in Chapter 4.

The current study examined the effects of ATN lesions on a modification of Kesner's non-spatial paired-associate tasks (Kesner et al., 2005). Some rats were trained on an odour-trace-object paired-associate task, but others were trained an odour-object paired associate task, without an explicit delay (trace) between the odour and object. We have shown in Chapter 6 that intact rats are able to acquire both variations of this paired-associate task. If the ATN and HPC are interdependent structures within the memory system, we would expect ATN lesions to remove the ability for rats to acquire the odour-trace-object task, because CA1 lesions prevent acquisition of this task and ATN lesions (and MTT lesions) are known to reduce microstructural integrity of CA1 neurons and impair memory for temporal order (Dillingham et al., 2019; Harland et al., 2014). In contrast, it was anticipated that ATN lesions would leave acquisition of the odour-object (No Trace) task intact or only transiently delay acquisition on the non-temporal version of this paired-associate task. Non-temporal paired-

associate object-odour tasks appear to be unaffected by hippocampal lesions (Gilbert & Kesner, 2002). In addition, IEG Zif268 expression was assessed after a retention test conducted 5 days after the end of training. We expected to see relative hypoactivation of IEG expression in the RSC following injury to the ATN. However, the task that included a trace (delay) component was shown (Chapter 6) to increase CA1 Zif268 expression in intact rats compared to the no-trace paired associate task (Chapter 6). We therefore also predicted reduced HPC CA1 activation of Zif268 in the ATN group, compared to the intact rats. This study also provided an opportunity to provide a replication of the increased CA1 activation of Zif268 in Sham rats when a trace component was included.

7.2 Materials and Methods

7.2.1 Subjects

Forty 12-month old male Long-Evans rats were used in this study. They were bred and housed in the University of Canterbury's animal facility. Housing conditions and food restriction procedures were the same as outlined in Chapter 5. All procedures carried out were approved by the Animal Ethics Committee of the University of Canterbury (#2018/10R).

7.2.2 ATN lesion surgery

Anaesthesia was achieved by placing the rat in an induction box with 4% isoflurane (Provet, NZ) with oxygen at a flow rate of 2000 ml/min. Once anaesthesia was achieved (no response to plantar or tail pinch) the rat was injected with Carprofen (subcutaneous 5mg/kg; Provet, NZ) for pain relief and Hartmann's solution (2ml intraperitoneal sodium lactate, Baxter, NZ). The head was shaved and cleaned, and the rat was placed back in the induction chamber briefly. The rat was then transferred to the nose cone on the stereotaxic apparatus. Methopt Forte (Aspen Pharm, NZ) eye drops were applied, and a damp gauze placed above and clear of the eyes. The incision site was cleaned twice with 4% chlorhexidine gluconate on sterilised

gauze and injected with local analgesia (subcutaneous Mepivacaine 0.02 ml of 2 mg/ml). Core temperature was maintained by insulating the rat in bubble wrap. Following this, plantar and tail pinch checks were performed every ten minutes to ensure maintenance of general anaesthesia. The rat was maintained at 2.5% isoflurane with oxygen (flow rate: 1500 ml/min) through the nose cone throughout the surgical procedure with all waste gas scavenged via an exhaust system into the outside atmosphere. If plantar or tail pinch response returned during surgery, the rat was put back on 4% isoflurane until the reflex disappeared. For these ATN lesion surgeries the incisor bar was set at -7.5mm below the interaural line to minimise fornix damage. Two infusions per hemisphere were directed at the anteroventral nucleus (AV; upper and lower) and one infusion per hemisphere was directed at the anteromedial nucleus (AM). Co-ordinates used for each site are shown in Table 7.1. Each surgery used one of five anterior-posterior coordinates relative to an individual rats Bregma-Lambda distance. Lesions were made first to all four AV sites, followed by the two AM sites. 0.15M N-methyl-d-aspartate (NMDA; Sigma, Castle Hill, NSW) in 0.1M phosphate buffer (pH 7.20) was infused into each site at a rate 0.04ul per minute via a 2.5µL Hamilton syringe (Reno, NV, USA) driven by a micro infusion pump (Stoelting, Wooddale, IL). Following infusion, the needle remained in situ for a further 3 minutes per site for diffusion of the NMDA. Sham lesion surgeries used the same procedure except that the needle was lowered to 1 mm above the lesion site with no infusion.

Table 7.1. ATN surgical co-ordinates

B-L Distance	AP	Lateral	DV from dura	Volume NMDA
AV				
≤ 0.64	-0.210	±0.152	Upper: -0.568	Upper: 0.12µl
0.65 – 0.68	-0.215		Lower: -0.573	Lower: 0.10µl
0.69 – 0.72	-0.220			
0.73 – 0.76	-0.225			
≥ 0.77	-0.230			
AM				
≤ 0.64	-0.200	±0.116	-0.576	0.06µl
0.65 – 0.68	-0.205			
0.69 – 0.72	-0.210			
0.73 – 0.76	-0.215			
≥ 0.77	-0.220			

Abbreviations: AM = anteromedial thalamic nuclei; AP = anterior-posterior co-ordinates; B-L = Bregma-Lambda distance; DV = dorsal-ventral (depth) co-ordinate; NMDA = 0.15M N-methyl-d-aspartate.

7.2.3 Behavioural Procedures

7.2.4 Radial arm maze

To verify the effects of ATN lesions, spatial working memory was examined in a radial arm maze (Figure 7.1). The maze (67.5 cm high) was located in the centre of a windowless room (3 x 3 metre). Stimuli (distal cues) were added to the walls, including posters, toy animals, small cones, a curtain, tables, and empty ice cream containers. The maze was made up of a 35 cm wide central grey hub with 8 aluminium arms (65 cm long by 8.6 cm wide, with 4.5 cm-high borders). Clear Perspex walls (19 cm long by 25 cm high) extended along one side of each arm from the central area to prevent rats from jumping between arms. A black wooden food receptacle (A, Figure 7.1; 5 cm x 8.5 cm x 3 cm), with inaccessible food underneath a small hole in the centre of the well, was placed at the end of all eight arms. For testing, food rewards (2 x 0.1g chocolate drops) were presented in each food well. Perspex guillotine doors (B, Figure 7.1), which could be raised up from beneath the hub by a pulley system under the maze, controlled access to the hub and arms. The experimenter sat in the corner of the room at a table to operate the pulley system (C, Figure 7.1). Each arm of the maze was at least 40 cm away from all walls and tables.

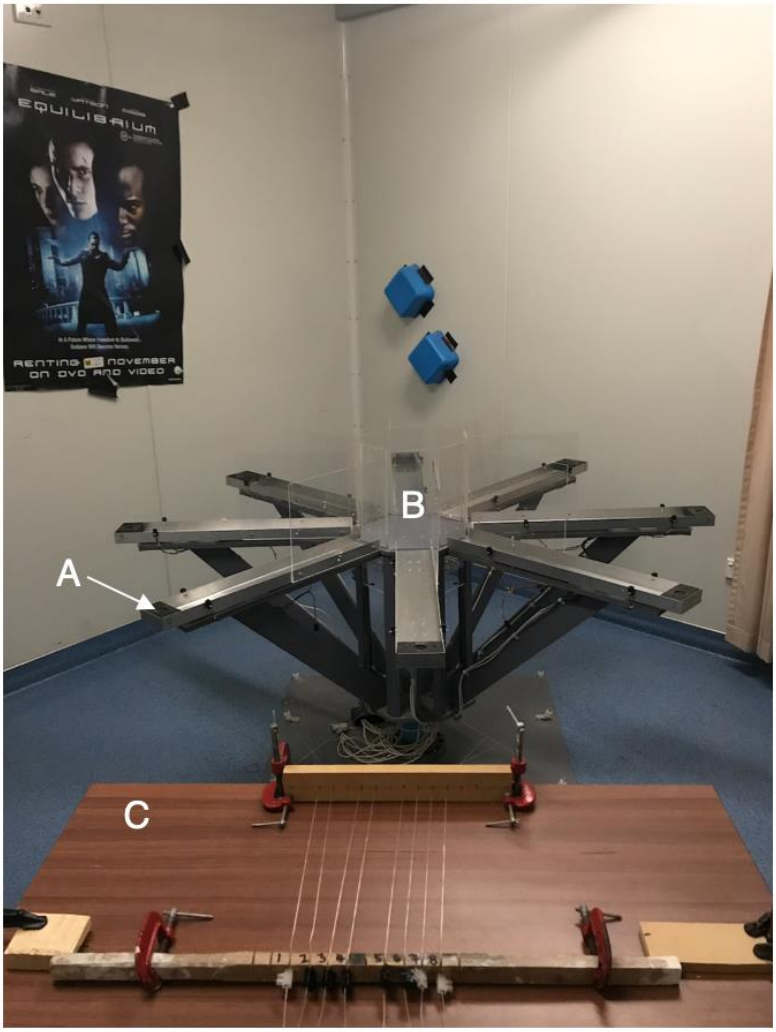

Figure 7.1. Radial arm maze used in the spatial working memory task. **A** = black wooden food well at the end of the arms; **B** = clear Perspex guillotine door; **C** = table with pulley system used to open the Perspex doors on the central hub of the maze.

Pre-surgery RAM habituation

Over two weeks, rats were food-restricted to maintain their body weight at 85% of ad libitum weight. They were handled for 5 minutes a day and habituated to the food reward in their home cages. Prior to lesion surgery, on approaching the 85% weight target, the rats were habituated to the RAM for seven days. Cage mates were placed in the maze for 10 minutes for three days with chocolate drops scattered lightly in the central hub and more densely down the arms and in the food wells. They were then habituated individually for five minutes a day with the

doors raised up and down at random intervals. Food rewards were moved further down each arm so that by the final day habituation the chocolate was present only in the food wells.

Radial arm maze procedure

Following re-familiarisation post-surgery, the rats received 10 consecutive days of testing in the standard RAM working memory task. Each food well at the end of the eight arms were baited only once per trial with two chocolate pellets. The rat was placed in the central hub and ~10 seconds later all eight arms were opened, and the rat allowed to make a choice. Choice behaviour was defined as all four limbs down an arm. Once the rat had entered an arm all the doors were then closed, and the rat was confined to the arm for ~15 seconds to eat the food reward. If the rat selected a previously entered arm, it was considered an error and the rat was confined to the arm for ~15 seconds. After the ~15 second delay the arm door was opened, and the rat was allowed back into the central hub. The rat was held in the central hub for ~10 seconds before all the doors were again opened to allow another arm selection. A trial concluded when the rat had visited all 8 arms, 20 arm choices had been made or 10 minutes had elapsed.

7.2.5 Simple discrimination and paired-associate tasks

The runway apparatus and general shaping and testing procedures was the same as previously described (Chapter 6). For reference, Figure 7.2 provides the schematic of the runway, as well as the new objects (C, short and tall pairings) used in the simple discrimination and paired-associate tasks.

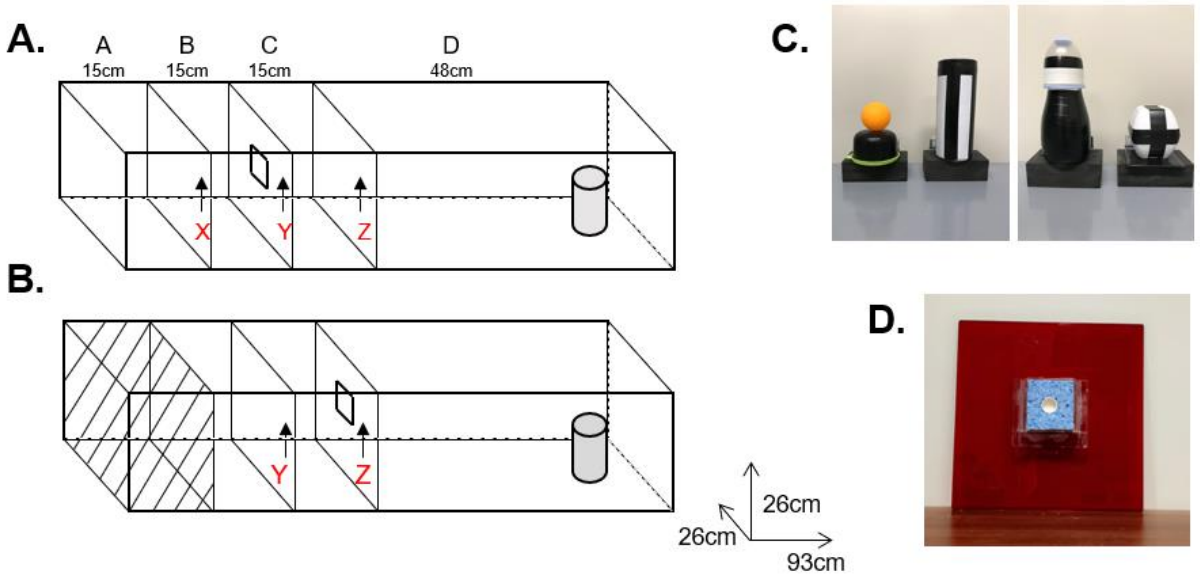

Figure 7.2. **A** and **B** replicate Figure 6.1 in Chapter 6: **A.** Placement of doors for the odour-trace-object paired associate task. **B.** Placement of doors for the odour-object paired associate task. **C.** Objects used in the simple (left) and paired associate (right) tasks. **D.** Odour receptacle and door used in the simple and paired associate tasks. For simple odour or simple object discrimination tasks, see Figure 5.1 legend (Chapter 5).

Odour/object pre-surgery shaping

Habituation and shaping procedures were the same as in Chapter 5.

Odour/object discrimination and association methods

Rats were randomly assigned to the No-Trace (ATN-No Trace n=10; Sham-No Trace, n=10) and Trace (ATN-Trace n=10; Sham-Trace, n=10) conditions. All tasks were run using the same 'go, no-go' procedure as in Chapter 6.

Simple odour and object discrimination tasks

Simple discrimination tasks were carried out as defined in Chapter 6. Rats were trained on the simple odour and simple object discrimination task until they reached a criterion of 80% correct over 2 consecutive days. These tasks used odours 1 (lemon) and 2 (clove) and objects A (short orange ball) and B (tall striped can; **C**, Figure 7.2), counterbalanced across rats.

Trace and no-trace odour-object association memory tasks

The odour-object and odour-trace-object paired-associate tasks were carried out as defined in Chapter 6. This task used odours 3 (cinnamon) and 4 (lime) and objects C (tall black bottle) and D (short white box; C, Figure 7.2), pairings of which were counterbalanced across rats. Rats always received a reward for the odour presented (one chocolate drop), but for example, then received a food reward (two chocolate drops) under Object C if Object C followed the presentation of Odour 3, but no reward if Object D instead followed Odour 3; conversely, Object D following Odour 4 would be rewarded but Object C following Odour 4 would not be rewarded.

Post-acquisition recall test

Recall of the paired-associate task (No Trace or Trace) was conducted 5 days either after the rat reached criterion or at the end of 50 days of formal training. This recall session used 12 massed trials in identical fashion and with the same odours and objects as used in training.

7.2.6 Histological procedures

Perfusion and tissue preparation

The Zif268 induction procedure was carried out as defined in Chapter 6. Perfusion procedure and collection of coronal brain sections were as outlined in Chapter 5.

Cresyl violet staining

Coronal slices were mounted from 0.1 M PB solution onto subbed slides and then air dried overnight. The slides were first delipidised in 70%, 95% and 100% ethanol for 10 dips each, with a subsequent 5 minutes in 100% ethanol and 10 dips in 95% ethanol, followed by 5 minutes in 70% ethanol. Slides were rehydrated in distilled H2O for 1 minute, before being placed into 4% cresyl violet acetate solution for 10-14 minutes. Slides were rinsed twice in distilled H2O for 2 minutes, before dehydration and differentiation: 70% ethanol and 95%

ethanol for 2 minutes, followed by 95% acid alcohol (solution of 400ml of 95% ethanol with 1ml glacial acetic acid) for 40 seconds, and 100% ethanol twice for 4 minutes. Slides were subsequently cleared in xylene twice for 5 minutes and cover slipped with DPX and left to dry.

NeuN staining

Following a similar procedure to Zif268 staining (Chapter 5), free floating sections through the ATN were processed with the antibody NeuN (specific to Neuronal Nuclei). Sections were washed in 0.1M PBSTx before being incubated in endogenous peroxidase blocking buffer for 30 minutes (1% hydrogen peroxide (H_2O_2), 50% methanol (CH_3OH) in 2% PBSTx). Sections were incubated overnight (24 hours) at 4˚C in anti-NeuN primary antibody (1:5000; Millipore) in PBSTx with 1% NGS. Excess antibody buffer was removed with PBSTx and followed by incubation in biotinylated goat anti-mouse secondary antibody (1:1000: Vector) overnight at 4 ˚C in PBSTx and 1% NGS. Sections the incubated in ExtrAvidin (peroxidase conjugated; 1:1000; Sigma), PBSTx and 1% NGS for 2 hours at room temperature. To remove excess ExtrAvidin and Triton X-100, the sections were washed in PBS, in PB, and then in Tris buffer (pH 7.4 in distilled H_20), to prepare the sections for visualisation with DAB. The DAB (0.05%; Sigma) with 0.01% H_2O_2 in Tris buffer reaction (approximately 5 minutes) was stopped using Tris buffer (1x 10 minute wash) and sections were placed in PB at 4˚C overnight before mounting onto gelatinised slides and allowed to dry. The slides were dehydrated through graded alcohol (70-100%) before being cleared in xylene and cover slipped with DPX.

Zif268 immunohistochemistry

Zif268 staining was done in the same manner as Chapter 5.

7.2.7 Regions of interest

Regions of interest (ROIs) were selected that had potential covert pathology related to ATN lesions and involvement in a No-Trace/Trace odour-object association memory (Figure 7.3). These regions included the hippocampus (dorsal: CA1, CA3 and dentate gyrus (DG); ventral: CA1 and CA3), subiculum (dorsal and ventral), medial prefrontal cortex (area 32V), anterior cingulate cortex (A32D and A24 areas 24a and 24b), all regions of the retrosplenial cortex (rostral and caudal A29 and A30), and the perirhinal and entorhinal cortices. The auditory cortex was analysed as a control control region where zif268 immunoreactivity would not be expected to differ following ATN lesions.

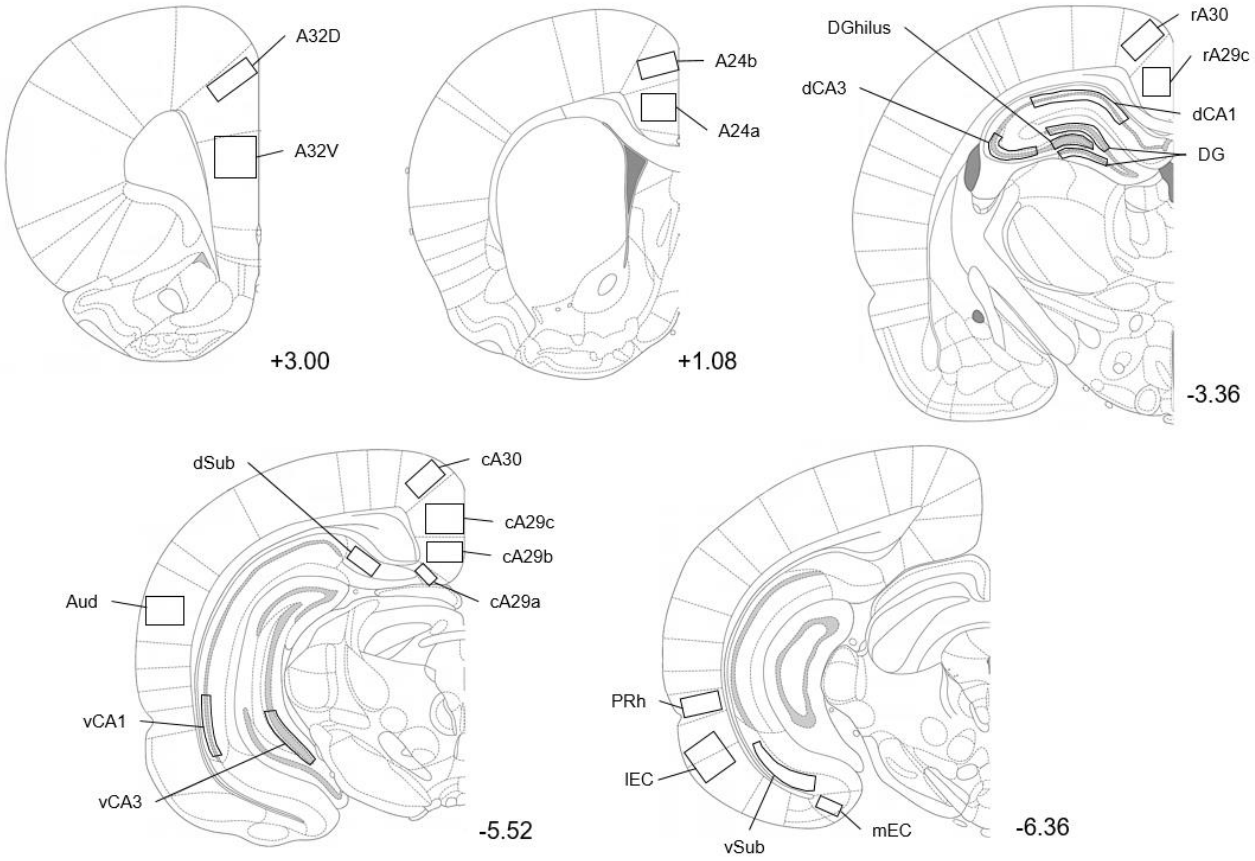

Figure 7.3. Regions of interest for Zif268 expression. A24a/b = area 24a/b of the anterior cingulate cortex; A32D/V = dorsal/ventral area 32 of the anterior cingulate/prefrontal cortex; Aud = primary auditory cortex; cA29a/b/c = caudal area 29a/b/c of the retrosplenial cortex; cA30 = caudal area 30 of the retrosplenial cortex; dCA1 = Cornu Ammonis Area 1 of the dorsal hippocampus; dCA3 = Cornu Ammonis Area 3 of the dorsal hippocampus; DG= dentate gyrus of the dorsal hippocampus; DGhilus = hilus of the dentate gyrus of the dorsal hippocampus; lEC = lateral entorhinal cortex; dSub = dorsal subiculum; mEC = medial entorhinal cortex; PRh = perirhinal cortex; rA29c = rostral area 29c of the retrosplenial cortex; rA30 = rostral area 30 of the retrosplenial cortex; vCA1 = Cornu Ammonis Area 1 of the ventral hippocampus; vCA3 = Cornu Ammonis Area 3 of the ventral hippocampus; vSub = ventral subiculum. Atlas plates adapted from Paxinos and Watson (2014).

7.2.8 ATN lesion verification

Cell loss in the ATN was used to determine lesion extent. To factor in the shrinkage of the tissue following ATN lesions, the average area showing ATN neurons was measured in sham rats. This used sections from both the left and right hemisphere at each AP (every second 40µm section through the ATN). The ROI was selected and measured using ImageJ (NIH, USA). Automated NeuN counts of ATN sparing relative to the relevant intact ATN sections were used to generate the number of cells spared from lesioning (cells/mm^2).

NeuN positive cell staining was identified and photographed at 5x objective with a light microscope (Leica, Germany). Automated counts of the cells were obtained through ImageJ (image analysis software, National Institute of Health, NIH, USA). The ROI was selected and the images were converted to 8-bit grey scale, background was subtracted (rolling = 40), converted to mask and the watershed function was applied, and all neuronal cells above threshold ('MaxEntropy' threshold, circularity 0.5-1) were counted. The detection threshold was the same for all sections. Total NeuN positive cell counts were averaged across the corresponding intact ATN (from Sham rats) sections to yield the number of ATN cells (per mm^2) spared in all lesioned animals. Acceptable lesions were defined as having less than 60% bilateral sparing, with no less than 30% damage to each hemisphere of the ATN.

7.2.9 Zif268 Quantification

Zif268 positive cell staining was identified and photographed with a 10x objective with a light microscope (Leica, Germany). Automated counts of the cells were obtained through ImageJ (NIH, USA). The ROI was selected and the images were converted to 8-bit grey scale, background was subtracted (rolling = 40), converted to mask and the watershed function was applied, and all Zif268 positive cells above threshold ('MaxEntropy' threshold, circularity 0.65-1) were counted. Counts of Zif268-positive cells for all regions used the same threshold algorithm, with between two to six sections per region of interest in each rat quantified. The average Zif268 positive cell count per mm^2 across sections (from both hemispheres) within an ROI was used.

7.2.10 Statistical analysis.

Statistical analyses were conducted using Statistica (v13; Dell Inc.). A reciprocal transformation of latency data in individual trials was used to ensure homogeneity of

variance. Reciprocal mean latency differences ('go' minus 'no-go' trial latencies (1/sec), per day) were used to assess discrimination between the different stimuli (simple discrimination) or pairings of stimuli (paired-associate tasks). Repeated measures ANOVA, one-way ANOVA and T-tests were conducted to test group difference in task acquisition, trials to criterion and Zif268 counts within and between ROIs. Where significant interactions were found, they were further explored through planned comparisons to establish simple main effects. Significance was set at $p<0.05$.

7.3 Results

7.3.1 Lesion verification and final sample sizes

17 of the 18 rats with lesions met the criterion of at least 60% (30% per hemisphere) bilateral damage to the ATN (average range of total lesion, 50.59-92.89%; Table 7.2). Rat D4 was excluded from analysis as there was incomplete bilateral damage, with a satisfactory lesion in one hemisphere only (75% left, 25% right). The smallest and largest included lesions are shown in Figure 7.4. Eight of the 40 experimental rats did not complete behavioural testing. Two ATN-lesioned rats died from surgical complications and six sham rats died prior to completion of behavioural testing (reasons unknown; two had post-mortem vet assessments, but no reason for death was identified). Consequently, these six rats were removed from data analysis (final n = 32 that completed all behavioural testing; Sham No Trace n = 7; ATN No Trace = 8; Sham Trace = 7; ATN Trace = 9).

Table 7.2. Percentage of ATN damage (left and right) as a result of NeuN cell loss of the included and excluded lesion rats.

INCLUDED	LEFT	RIGHT	ATN (average)
A3	82.80	97.45	90.13
B2	81.03	96.49	88.76
B3	38.26	94.03	66.15
C1	92.21	66.35	79.28
D1	85.26	96.12	90.69
D2	93.82	91.96	92.89
E1	45.69	94.28	69.98
E3	58.66	75.48	67.07
E4	96.68	38.20	67.44
F3	74.26	53.28	63.77
G2	33.54	89.62	61.58
G4	72.26	83.05	77.65
H2	33.09	90.05	61.57
H3	61.41	39.77	50.59
I3	74.50	78.43	76.47
I4	85.37	98.49	91.93
J1	81.73	37.28	59.51
Average	70.03	77.67	73.85
EXCLUDED			
D4	25.84	75.11	50.48

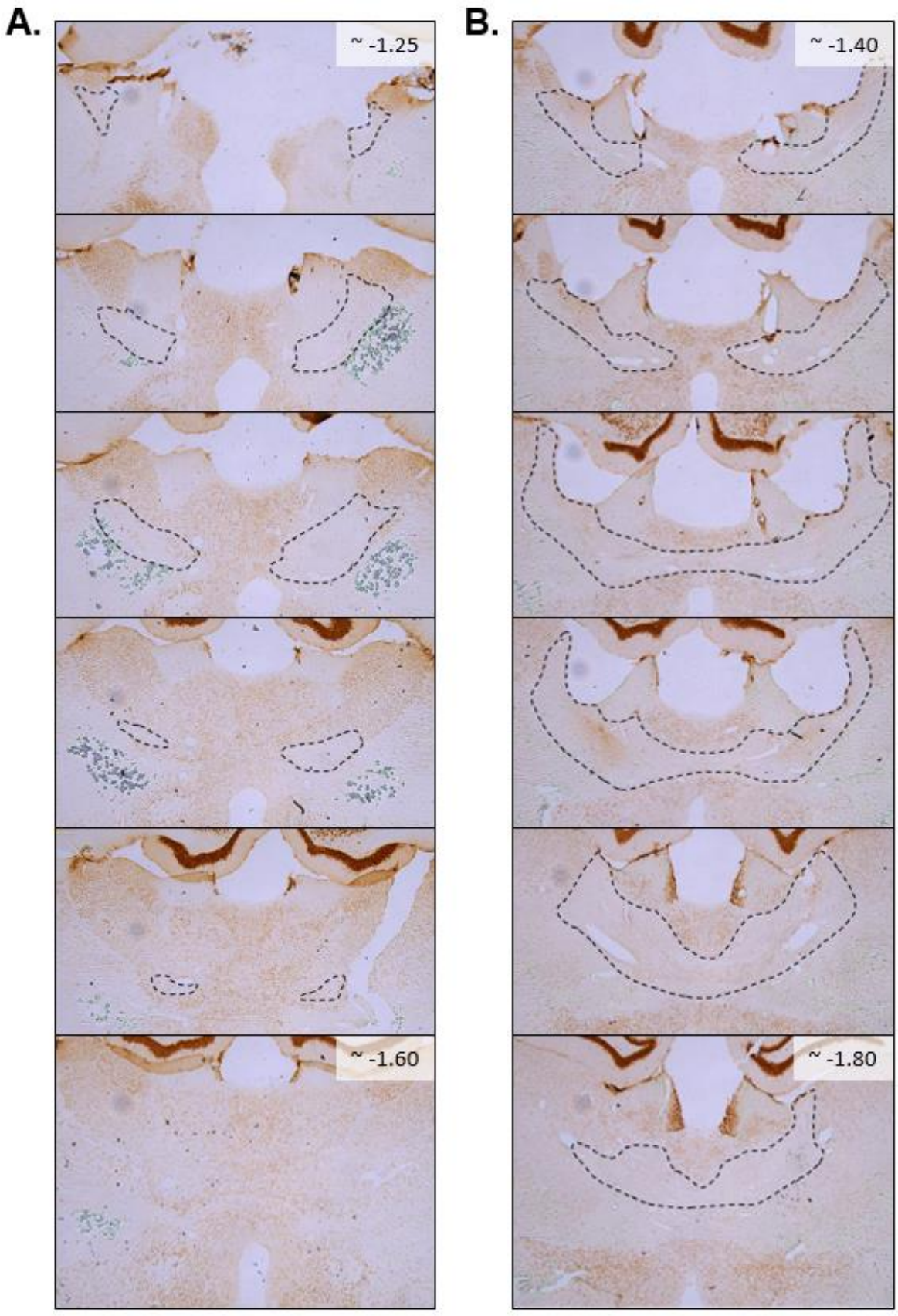

Figure 7.4. Photograph examples of the smallest (A.) and largest (B.) lesions in NeuN stained sections through the ATN. Numbers correspond roughly to the Anterior-Posterior location of the sections.

7.3.2 Spatial working memory in the radial arm maze (RAM)

As expected, rats with ATN lesions showed profoundly impaired acquisition of the spatial working memory task (Figure 7.5). Initial formal testing is due to many rats taking 10 minutes but relatively few arm entries. The performance of ATN and Sham group diverged on day two of training and the ATN group showed little evidence of improvement by day 10 (Group main effect, $F(1,29)=122.65$, $p<0.001$; Day, $F(9,261)=11.72$, $p=0.11$; Day x Group interaction, $F(9,261)=11.72$, $p<0.001$). ATN-lesion rats also made fewer correct arm choices before the first error than the sham group (Group main effect, $F(1,29)=75.95$, $p<0.001$; Day $F(9,261)=1.21$, $p=0.28$; Day x Group interaction, $F(9,261)=4.61$, $p<0.001$).

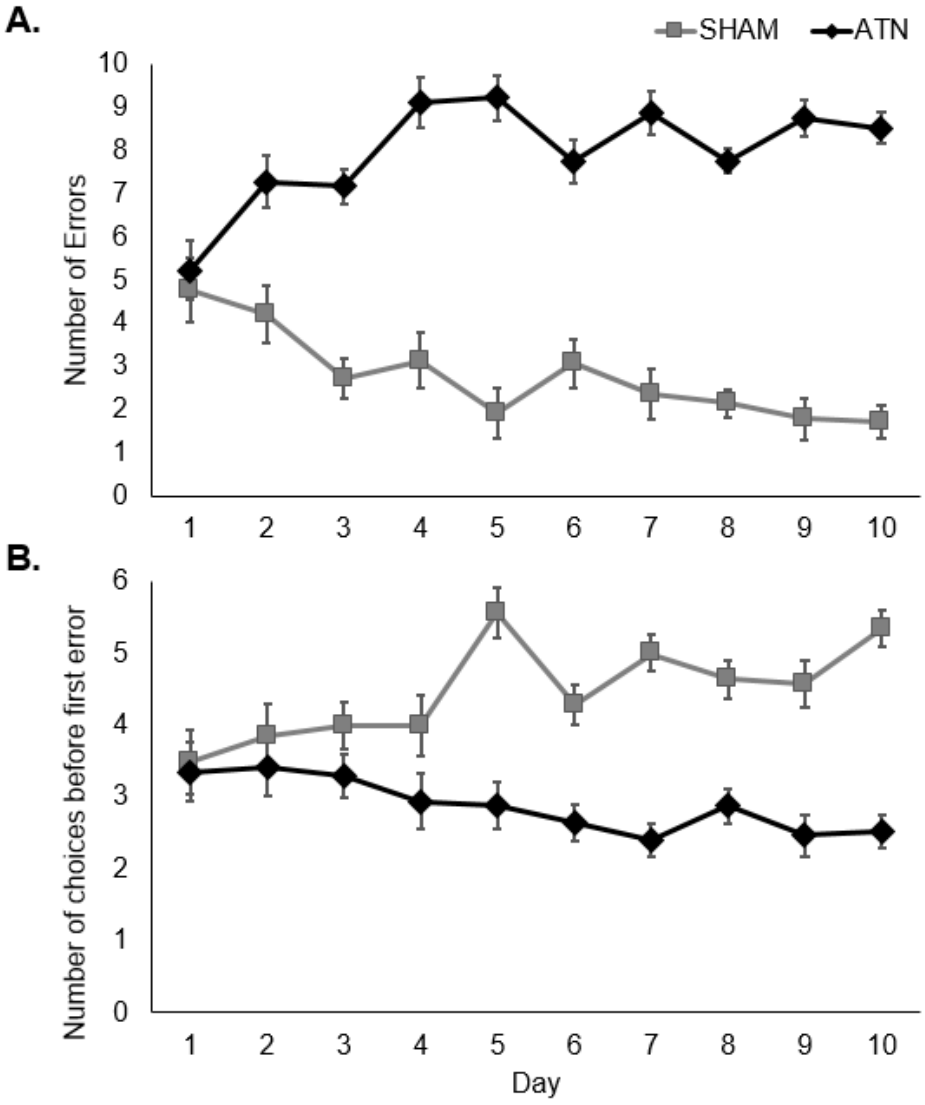

Figure 7.5. Mean (± SEM) spatial working memory errors per day (**A**) and number of arm choices before the first error (**B**) on the standard RAM task post lesion surgery.

7.3.3 Simple discrimination tasks in the runway

Both Sham and ATN-lesion groups rapidly acquired the simple odour discrimination task (**A**, Figure 7.6; Group main effect, $F(1,29)=0.06$, $p=0.80$; Day x Group interaction, $F(5,145)=0.43$, $p=0.82$). Although more variable across training, there was also no difference in performance between Sham and ATN-lesion groups on the simple object discrimination task (**B**, Figure 7.6; Group main effect, $F(1,29)=1.03$, $p=0.31$; Day by Group interaction, $F(6,174)=1.12$, $p=0.34$). Rats took an average of 4 to 5 days to reach criterion on the odour discrimination and 5 to 6 days for the object discrimination task (odour: Sham M=4.64,

SD=0.84; ATN M=4.35, SD=0.93, t=0.90, p=0.37; object: Sham M=5.57, SD=0.85; ATN M=5.29, SD=1.04, t=0.79, p=0.43).

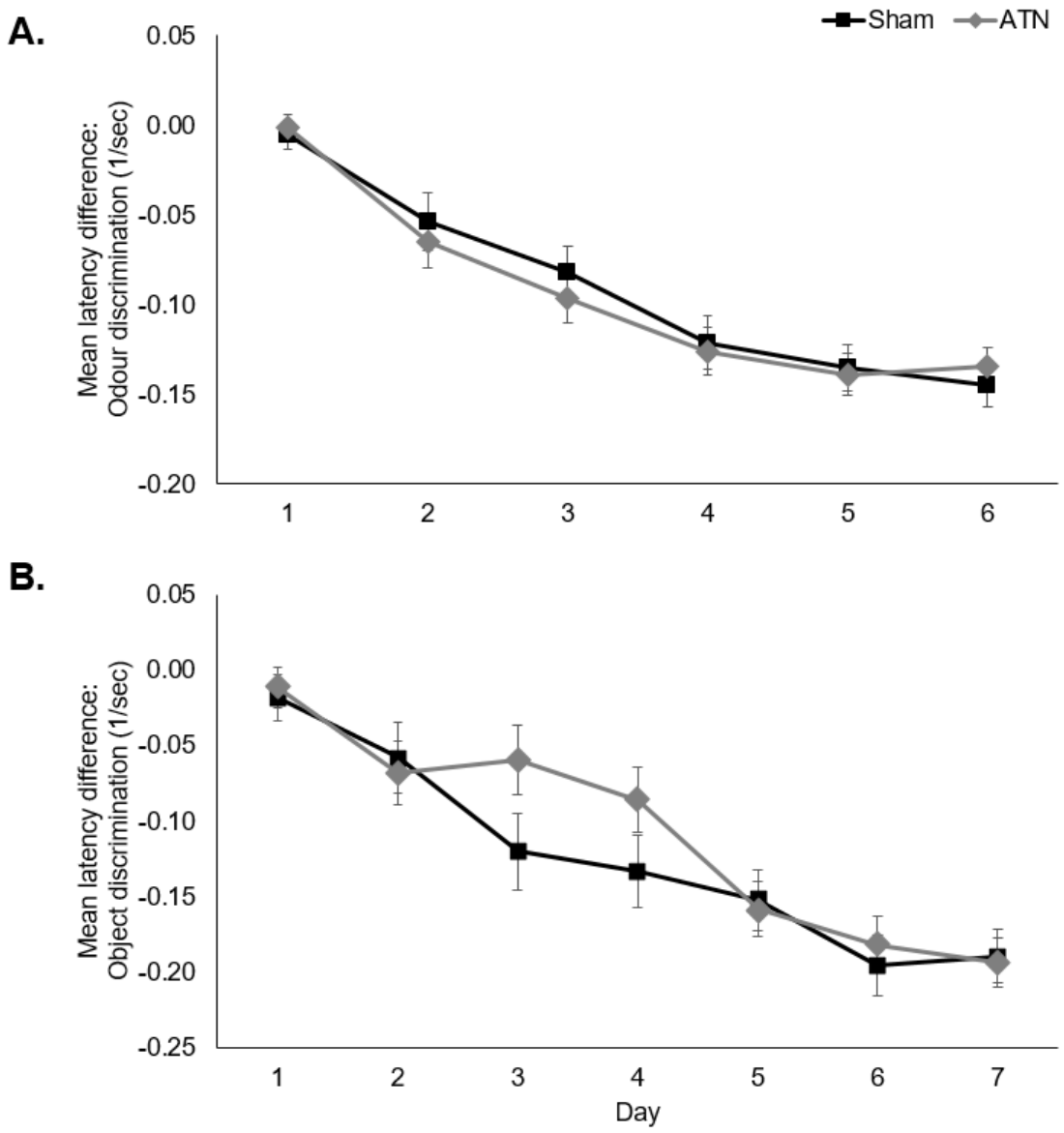

Figure 7.6. Task acquisitions of the A. simple odour discrimination and B. simple object discrimination (reciprocal mean latency difference, ±SEM). ATN = anterior thalamic lesion group; Sham = Sham lesion group.

7.3.4 No-Trace and Trace odour-object paired-associate tasks

Task acquisition measured by the mean latency difference (latency of incorrect paired trial minus latency of correct paired trials; latency = 1/second) for both Trace conditions (odour-object without trace; odour-trace-object) is shown in Figure 7.7. Rats in the trace (delay)

condition showed faster acquisition of the paired-associate task than rats trained in the No Trace condition (Trace main effect, $F(1,25)=5.78$, $p=0.02$). However, ATN lesions removed the ability to acquire both paired-associate tasks (Lesion main effect, $F(1,27)=151.24$, $p<0.001$; Block x Lesion $F(9,243)=69.72$, $p<0.001$). The Trace by Lesion interaction ($F(1,27)=3.51$, $p=0.07$) and the Block by Trace by Lesion interaction ($F(9,243)=1.21$, $p=0.28$) were not significant. These analyses suggest faster acquisition in the Trace relative to the condition for the Sham-lesion groups.

The Sham-lesion groups retained good discrimination between correct and incorrect pairings on the 5-day retention test, but now Sham-lesion Trace groups performance was significantly better than the Sham-lesion No-Trace group (Lesion main effect, $F(1,27)=268.27$, $p<0.001$; Trace main effect, $F(1,27)=6.19$, $p=0.01$; Lesion x Trace interaction, $F(1,27)=5.76$, $p=0.02$). Analysis of the simple main effects: Sham-lesion Trace versus Sham-lesion No Trace ($F(1,27)=10.91$, $p=0.002$), with clear deficits in both ATN-lesion conditions (ATN-lesion Trace versus Sham-lesion Trace, $F(1,27)=181.16$, $p<0.001$; ATN-lesion No Trace versus Sham-lesion No Trace $F(1,27)=95.16$, $p<0.001$). Performance for all groups did not change in the 5-day retention test relative to the end of training (Block 10; Block main effect, $F(1,27)=0.08$, $p=0.76$; Block x Trace, $F(1,27)=0.02$, $p=0.88$; Block x Lesion, $F(1,27)=0.10$, $p=0.74$; Block x Trace x Lesion interaction, $F(1,27)=0.53$, $p=0.47$).

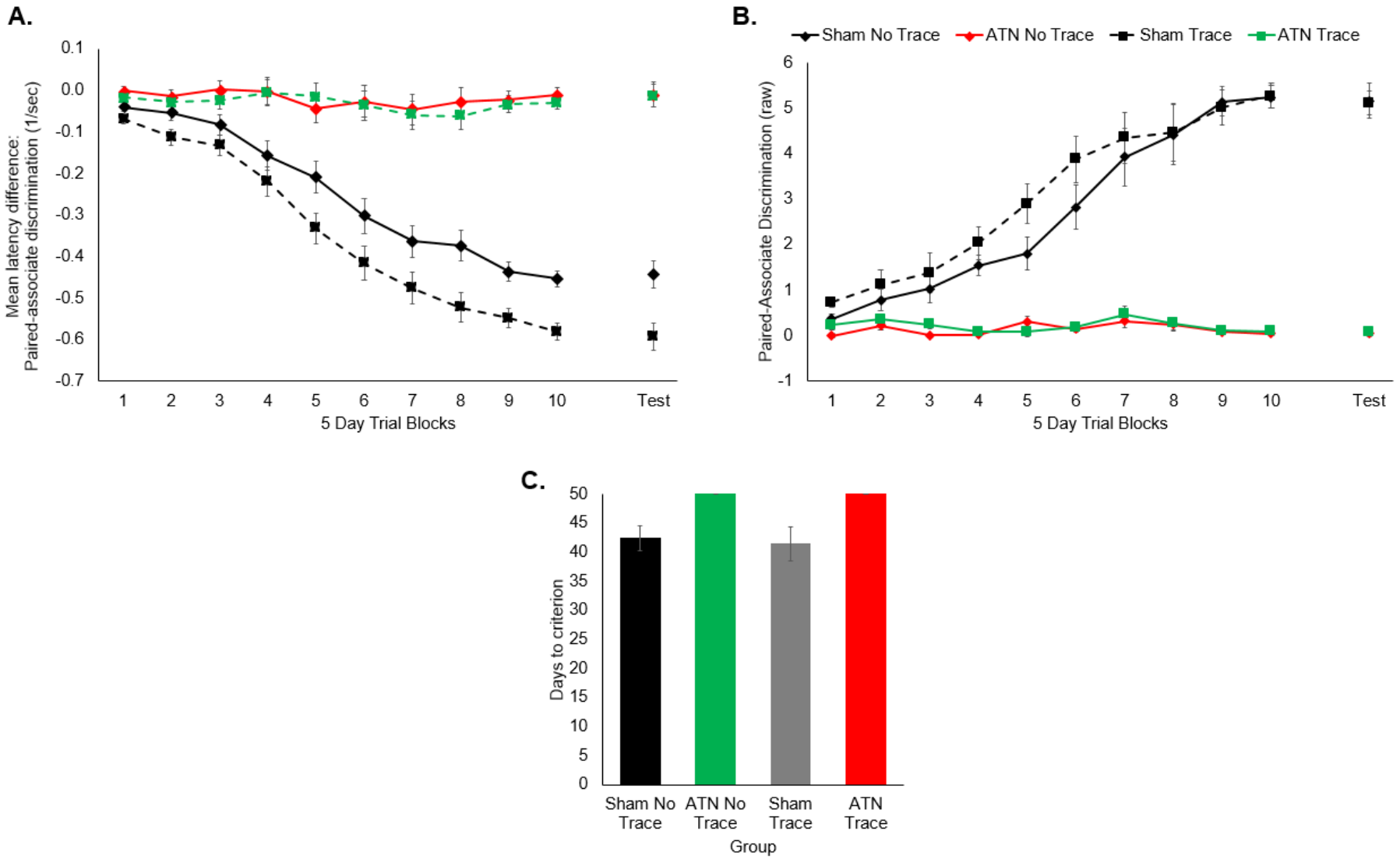

Figure 7.7. Acquisition and retention for Sham and ATN lesioned rats on the odour-object and odour-trace-object paired-associate memory task in 5-day trial blocks. **A.** Normalised acquisition latency scores; mean latency difference of 0.00 represents no difference, and -0.50 represents approximately a 5 second difference in responding to the 'go' and 'no-go' trials. **B.** Raw mean latency difference discrimination scores in seconds. **C.** Average days to criterion for each group. Error bars = standard error.

Additional analyses were conducted on correct (Figure 7.8, **A**, ‘go’) and incorrect (Figure 7.8, **B**, ‘no-go’) trials, separately, to assess running speeds in more detail. On ‘go’ trials (correct pairings), rats in the Trace condition responded faster to the object than rats in the No Trace condition (Trace main effect $F(1,27)=6.77$, $p=0.01$). ATN lesion rats in both conditions were slower to respond on correct trials compared to Sham lesion rats (Lesion main effect $F(1,27)=10.21$, $p=0.003$). There was no Trace by Lesion interaction ($F(1,27)=0.71$, $p=0.40$; Lesion x Trace x Trial Block $F(9,243)=0.644$, $p=0.75$).

In the ‘no-go’ trials, sham lesion rats showed slowed responding, while ATN lesion rats did not (Lesion main effect $F(1,27)=65.25$, $p<0.001$). There was no difference in responding between the Trace conditions on the ‘no-go’ trials (Trace main effect $F(1,27)=0.95$, $p=0.33$). Across the trial blocks, rats in the Sham groups (both No Trace and Trace) decreased response latency to these incorrect pairings, whereas ATN-lesion rats increased their latencies (Trial Block x Lesion interaction $F(9,243)=76.91$, $p<0.001$).

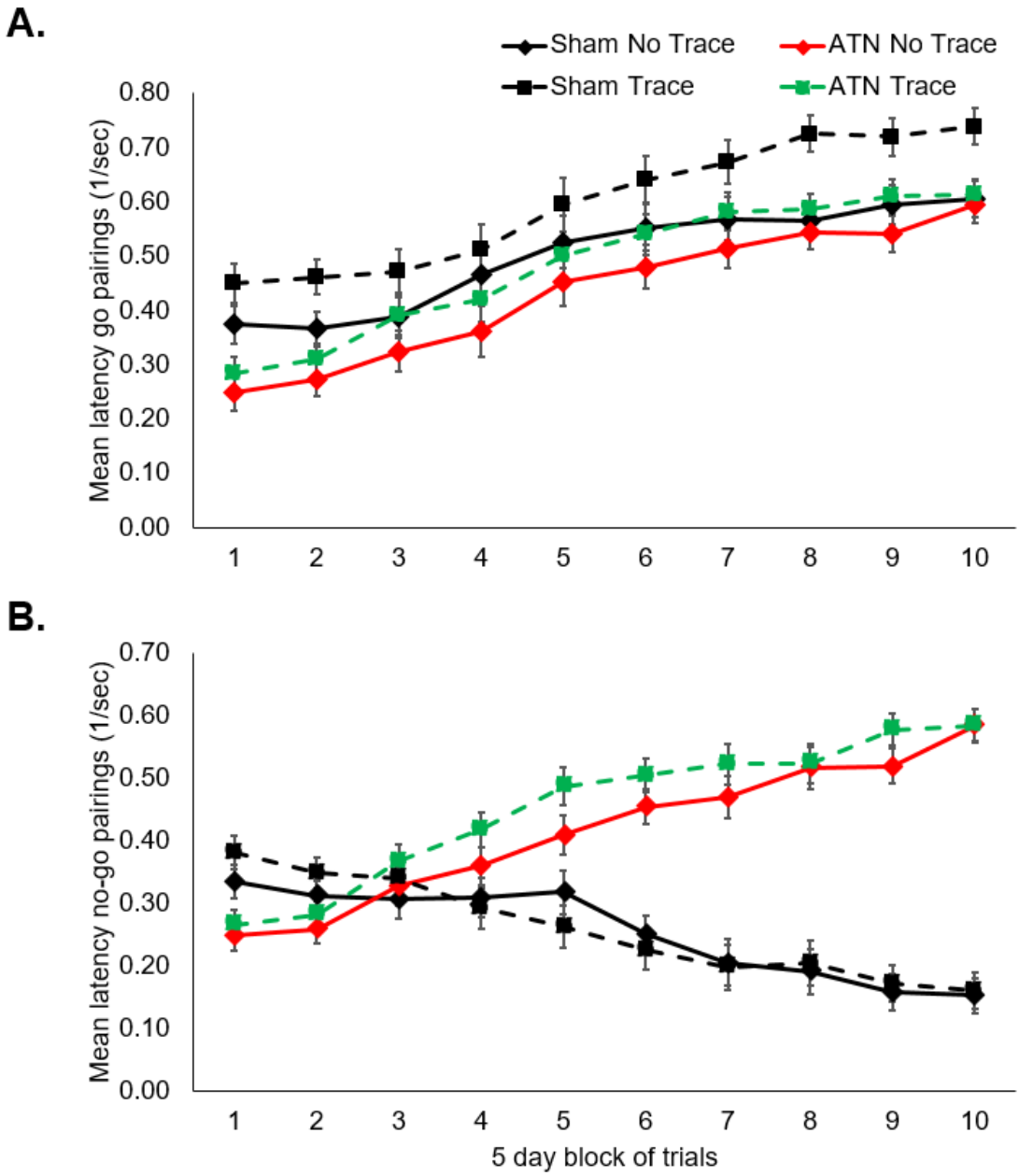

Figure 7.8. Mean latency (reciprocal: 1/sec) of 'go' (**A**) and 'no-go' (**B**) pairings across acquisition of the odour-object and odour-trace-object paired-associate tasks. Mean latencies were averaged across 5-day trial blocks.

7.3.5 Zif268 expression

Zif268 counts were analysed across ROIs in the four experimental groups following the retention test. Significant interactions of Trace and Lesion conditions were limited to the dorsal HPC. Trace main effects were found in the dorsal HPC, medial prefrontal cortex and both rostral and caudal A30 regions of the retrosplenial cortex. Lesion group effects were

found throughout all ROIs, except the entorhinal cortex, ventral HPC and subiculum and the auditory (control) cortex.

Hippocampus

Expression of Zif268 in the dorsal and ventral HPC and subiculum following the retention test 5-days after acquisition is shown in Figure 7.9. In the dorsal HPC, Zif268 expression was significantly higher in the CA1 than in other HPC subregions (dCA1; Subregion main effect $F(3,75)=668.97$, $p<0.001$). More importantly, there is a clear increase of expression for Sham-lesion rats for the Trace compared to No Trace conditions that depended on Subregion (Subregion x Trace x Lesion interaction $F(3,75)=8.39$, $p<0.001$). Further analysis of simple interaction effect for the dCA1 (Sham Trace x ATN Trace $F(1,25)=20.76$, $p<0.001$), the Sham Trace x Sham No Trace was significant ($F(1,25)=5.87$, $p=0.02$), reflecting the increase in Xif268 expression for CA1 in Sham-lesion rats in the Trace condition. Conversely, ATN lesions significantly reduced expression in the CA1 when there was a Trace. However, Sham and ATN groups did not differ for the CA1 when there was No Trace (Lesion x No Trace $F(1,25)=0.03$, $p=0.84$). ATN lesions reduced Zif268 expression when there was a Trace compared to No Trace, but this pair-wise comparison did not reach significance ($F(1,25)=3.41$, $p=0.07$).

Sham rats expressed higher counts of Zif268 when there was a Trace, but this difference was not replicated in ATN Trace and No Trace rats (Trace x Lesion interaction $F(1,25)=8.62$, $p=0.007$; simple interaction effect of Trace in: Sham, $F(1,25)=5.89$, $p=0.02$ ATN, $F(1,25)=2.81$, $p=0.10$). There was no main effect of Trace across the dorsal HPC ($F(1,25)=0.60$, $p=0.44$). Sham rats showed consistently higher counts of Zif268 across the dorsal HPC but this was only significant in the dCA1 (Lesion main effect $F(1,25)=7.82$, $p=0.009$; Lesion x Subregion interaction, $F(3,75)=10.72$, $p<0.001$; simple interaction effect of

Lesion: CA1, F(1,25)=11.02, p=0.002; CA3, F(1,25)=0.57, p=0.45); DG, F(1,25)=3.24, p=0.08; DGhilus, F(1,25)=0.06, p=0.80).

Neither Trace condition nor Lesion group altered Zif268 expression across the ventral HPC (Trace main effect F(1,26)=0.54, p=0.46; Lesion F(1,26)=2.55, p=0.12; Trace x Lesion interaction F(1,26)=0.34, p=0.56). The vCA1 had higher Zif268 expression than the ventral CA3 (Subregion, F(1,26)=124.36, p<0.001) but there were no Subregion interactions between the groups in the ventral HPC (Subregion x Trace interaction F(1,26)=1.08, p=0.30; Subregion x Lesion F(1,26)=0.55, p=0.46; Subregion x Trace x Lesion F(1,26)=1.39, p=0.24).

ATN lesions also reduced Zif268 expression in the subiculum (Lesion F(1,27)=3.96, p=0.05). There were also no Trace or Trace x Lesion effects in the subiculum (Trace main effect, F(1,27)=0.063, p=0.80; Trace x Lesion interaction, F(1,27)=0.26, p=0.61). There were higher counts in the ventral subiculum (F(1,27)=9.02, p=0.005), but there were no dorsal versus ventral Subregion interactions (Subregion x Trace interaction F(1,27)=0.03, p=0.84; Subregion x Lesion F(1,26)=0.37, p=0.54; Subregion x Trace x Lesion F(1,26)=0.03, p=0.86).

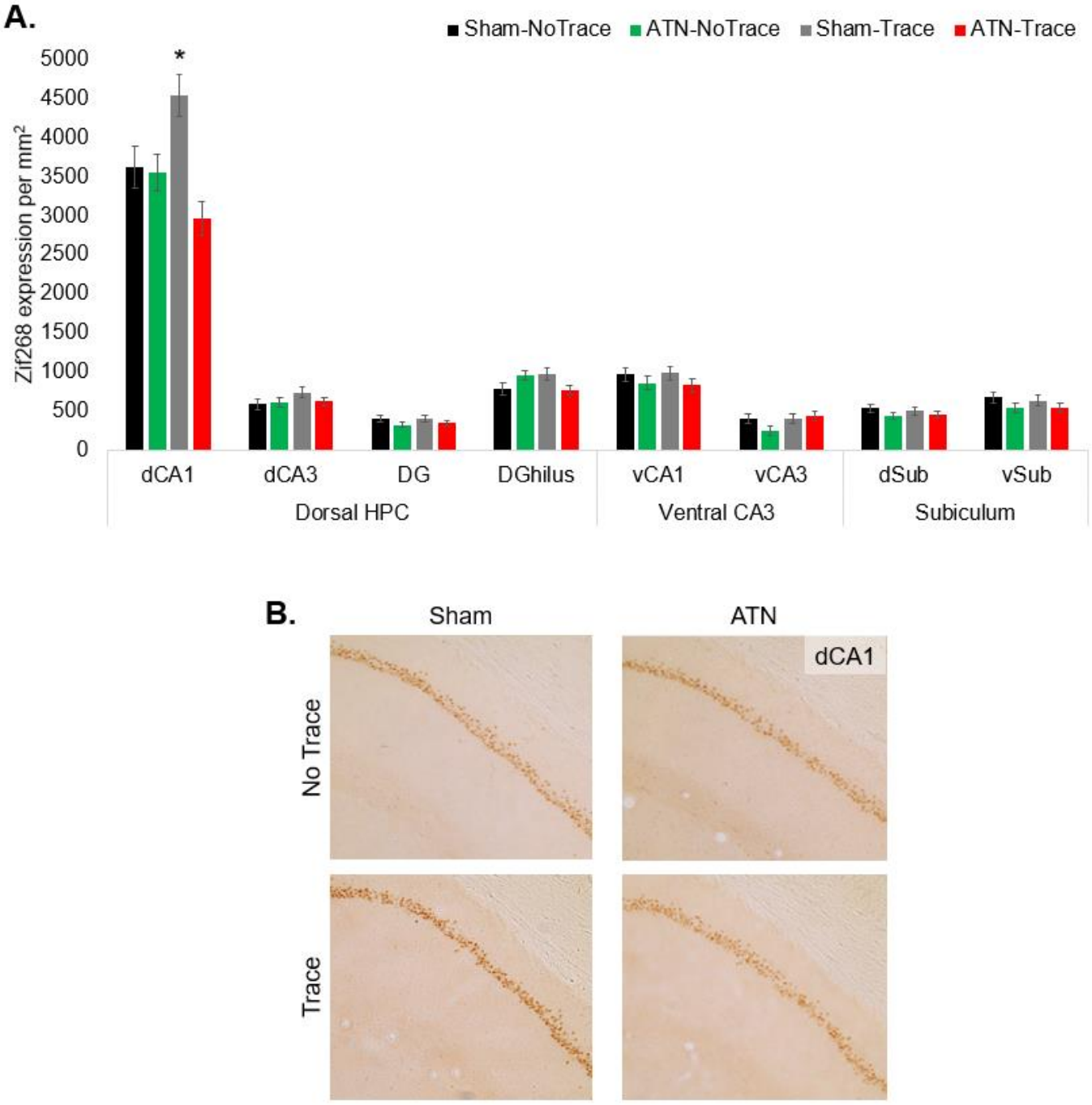

Figure 7.9. Zif268 expression per mm^2 in the hippocampal formation following the 5-day retention test. **A.** Mean (± Std. Err) hippocampal and subicular expression/mm^2. **B.** 10x magnification photomicrograph examples of Zif268 expression in the dorsal CA1 subregion; the examples represent a median case in each group. dCA1 = Cornu Ammonis Area 1 of the dorsal hippocampus; dCA3 = Cornu Ammonis Area 3 of the dorsal hippocampus; DG= dentate gyrus of the dorsal hippocampus; DGhilus = hilus of the dentate gyrus of the dorsal hippocampus; dSub = dorsal subiculum; vCA1 = Cornu Ammonis Area 1 of the ventral hippocampus; vCA3 = Cornu Ammonis Area 3 of the ventral hippocampus; vSub = ventral subiculum.

Prefrontal and anterior cingulate cortices

Zif268 expression in the medial prefrontal and anterior cingulate regions following the 5-day post-acquisition retention test is shown in Figure 7.10. ATN lesions clearly reduced zif268 expression in all regions of the prefrontal cortex.

ATN lesions significantly reduced Zif268 expression in A32D (Lesion main effect, $F(1,27)=19.65$, $p<0.001$). The greatest reduction in Zif268 following ATN lesions was seen in Layer II (Layer x Lesion interaction, $F(2,54)=3.79$, $p=0.02$; Layer II simple main effect $F(1,27)=33.33$, $p<0.001$, Layer III $F(1,27)=11.28$, $p=0.002$; Layer V $F(1,27)=7.60$, $p=0.01$). There was no difference seen between the two trace conditions (Trace main effect, $F(1,27)=1.11$, $p=0.30$) or any interactions involving Trace in A32D (Trace x Lesion interaction ($F(1,27)=0.13$, $p=0.72$; Layer x Trace interaction $F(2,54)=1.06$, $p=0.35$; Layer x Trace x Lesion $F(2,54)=0.22$, $p=0.79$).

ATN lesions significantly reduced expression in A32V of the prefrontal cortex (Lesion main effect, $F(1,27)=12.08$, $p=0.001$) but no Layer x Lesion interaction ($F(3,81)=0.05$, $p=0.98$). Rats showed higher expression when there was a Trace compared to No Trace ($F(1,27)=4.43$, $p=0.04$). Despite this, there was no significant interactions with Trace (Trace x Lesion interaction, $F(1,27)=0.46$, $p=0.50$; Layer x Trace interaction, $F(3,81)=1.60$, $p=0.19$; Layer x Trace x Lesion, $F(3,81)=1.87$, $p=0.14$).

ATN lesion rats showed marked reductions in Zif268 expression in the A24a cingulate cortex (Lesion main effect, $F(1,27)=33.30$, $p<0.001$). The greatest reduction in Zif268 expression with ATN lesions was in the two superficial layers (Layer x Lesion interaction $F(2,54)=3.69$, $p=0.03$), but present nonetheless in all layers (simple main effect of Lesion in Layer II, $F(1,27)=28.69$, $p<0.001$; Layer III $F(1,27)=14.61$, $p<0.001$; Layer V $F(1,27)=14.36$,

$p<0.001$). There was, however, no clear effect of Trace (Trace main effect, $F(1,27)=1.03$, $p=0.31$) or interactions with Trace in A24a (Trace x Lesion interaction, $F(1,27)=0.40$, $p=0.52$; Layer x Trace, $F(2,54)=0.96$, $p=0.38$; Layer x Trace x Lesion, $F(2,54)=1.43$, $p=0.24$).

ATN lesions produced reductions in 24b (Lesion main effect $F(1,27)=14.49$, $p<0.001$). However, these effects appeared not to be as substantial in A24b compared to A24a as lesions reduced expression in the superficial layers only (Layer x Lesion interaction $F(2,54)=4.58$, $p=0.01$; simple main effect of Lesion: Layer II, $F(1,27)= 20.52$, $p<0.001$, Layer III, $F(1,27)=8.68$, $p=0.006$, Layer V, $F(1,27)=2.48$, $p=0.12$). Zif268 expression was similar across the two Trace conditions in A24b and there was no interaction between Lesion and Trace (Trace main effect, $F(1,27)=1.49$, $p=0.23$; Trace x Lesion interaction, ($F(1,27)=1.04$, $p=0.31$). Despite no main effects of Trace, the superficial Layer II showed greater expression when there was a Trace compared to No Trace (Layer x Trace interaction $F(2,54)=3.69$, $p=0.03$; simple main effect of Trace in: Layer II, $F(1,27)=6.42$, $p=0.01$; Layer III, $F(1,27)=0.01$, $p=0.90$; Layer V, $F(1,27)=0.31$, $p=0.58$). There was no Layer by Trace by Lesion interaction ($F(2,54)=0.11$, $p=0.89$).

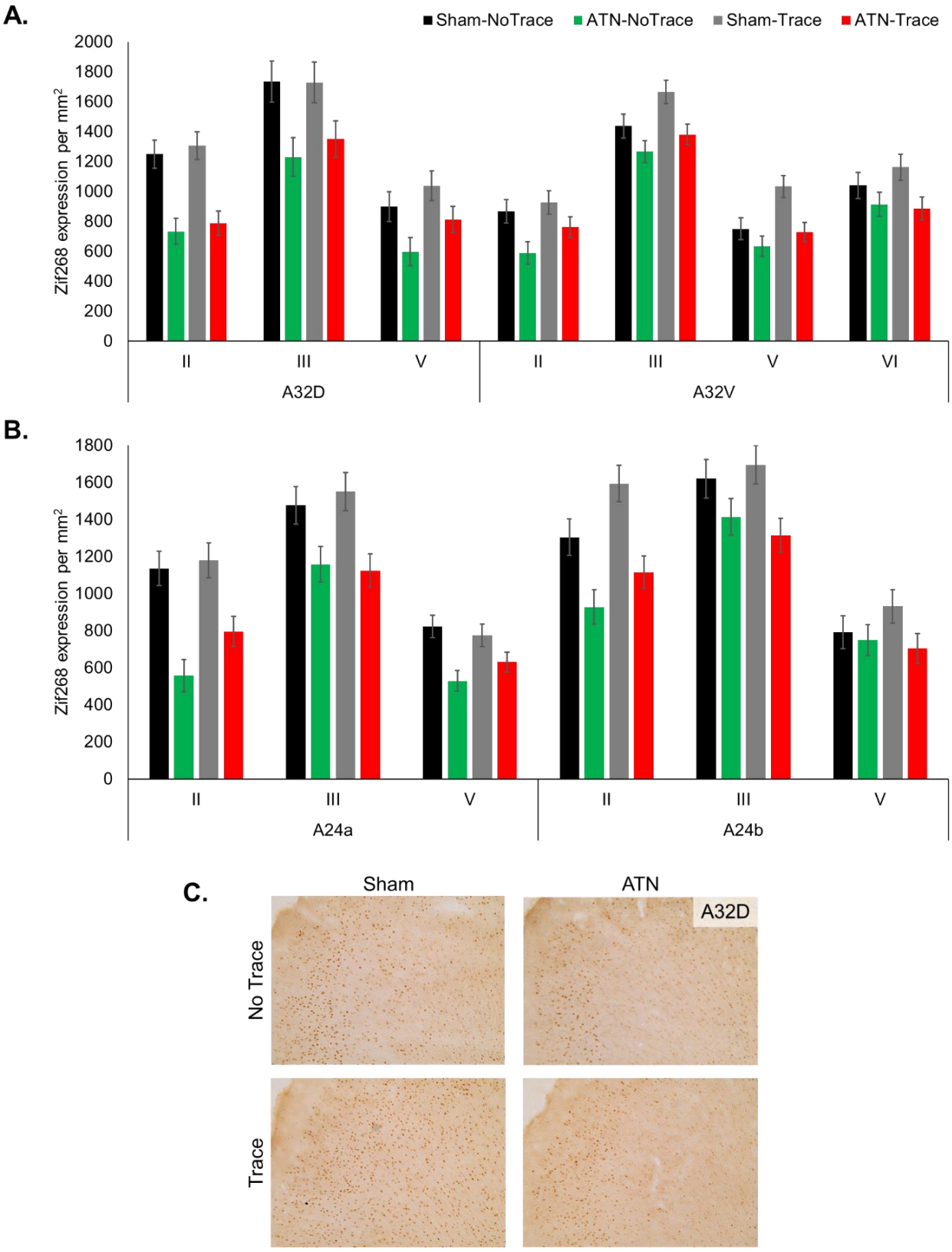

Figure 7.10. Zif268 expression per mm^2 in the medial prefrontal and anterior cingulate regions following the 5-day retention test. **A.** Mean (± Std. Err) Zif268 expression/mm^2 of the dorsal and ventral A32 regions. **B.** Mean (± Std. Err) Zif268 expression/mm^2 of A24a and A24b cingulate regions. **C**. 10x magnification photomicrograph examples of Zif268 expression in the A32D, examples represent the mean from each group. A24a/b = area 24a/b of the anterior cingulate cortex; A32D/V = dorsal/ventral area 32 of the anterior cingulate/prefrontal cortex.

Retrosplenial cortex

As expected, Zif268 expression in both rostral and caudal RSC was markedly reduced by ATN lesions, often more so in the superficial layers (Figure 7.11). ATN lesions produced a significant reduction of Zif268 expression in rostral A29c (rA29c; Lesion main effect $F(1,27)=104.43$, $p<0.001$). ATN lesions reduced cell counts more markedly in the superficial layers than the deeper layers (Layer x Lesion interaction $F(1,27)=50.91$, $p<0.001$; simple interaction effect of lesion: Superficial $F(1,27)=90.76$, $p<0.001$, Deep $F(1,27)=66.65$, $p<0.001$). The significant decrease in expression between the superficial and deep layers in Sham rats ($F(1,27)=203.00$, $p<0.001$) was also found in rats with ATN lesions ($F(1,27)=25.65$, $p<0.001$). There was no effect of trace condition on expression (Trace main effect $F(1,27)=0.57$, $p=0.45$; Trace x Lesion interaction $F(1,27)=0.97$ $p=0.33$; Layer by Trace interaction ($F1,27)=0$, $p=0.95$).

A similar pattern of expression was seen in rostral A30 (rA30), with ATN lesions significantly reducing Zif268 expression (Lesion main effect, $F(1,27)=104.61$, $p<0.001$). This lesion main effect was apparent across both layers of rA30 (Layer x Lesion interaction, $F(1,27)=62.16$, $p<0.001$; simple interaction effect of Lesion: Superficial $F(1,27)=99.33$, $p<0.001$, Deep $F(1,27)=62.45$, $p<0.001$). As with rA29c, the difference in expression across the two layers was more apparent in Sham than ATN lesion rats (simple interaction effect of Layer: Sham, $F(1,27)=388.10$, $p<0.001$, ATN, $F(1,27)=99.00$, $p<0.001$). Interestingly, rats expressed higher Zif268 counts in rA30 when there was a Trace, but only in the superficial layer (Trace main effect $F(1,27)=4.44$, $p=0.04$; Layer x Trace interaction $F(1,27)=5.14$, $p=0.03$; simple interaction effect of Trace: Superficial $F(1,27)=5.36$, $p=0.02$, Deep $F(1,27)=1.11$, $p=0.30$). There was no Layer by Trace by Lesion interaction ($F(1,27)=0.10$, $p=0.75$).

ATN lesions also reduced Zif268 expression of the caudal A29 regions (cA29) with the greatest reductions seen in cA29b and cA29c (Lesion main effect; $F(1,27)=116.28$, $p<0.001$; Subregion x Lesion interaction $F(2,54)=4.80$,$p=0.01$; Sham vs ATN lesion simple main effect in cA29a $F(1,27)=30.86$, $p<0.001$, cA29b $F(1,27)=98.87$, $p<0.001$, cA29c $F(1,27)=82.43$, $p<0.001$). These decreases in expression were more pronounced in the superficial layer of the three regions than in the deeper layer (Layer x Lesion interaction $F(1,27)=101.62$, $p<0.001$; simple main effect of lesion, Superficial Layer $F(1,27)=120.83$, $p<0.001$, Deep Layer $F(1,27)=48.01$, $p<0.001$).Trace did not alter cell counts in A29c (Trace main effect $F(1,27)=2.10$, $p=0.15$; Trace x Lesion interaction $F(1,27)=1.14$, $p=0.29$). There was however, an increase in Zif268 expression in the superficial layer when there was a Trace (Layer x Trace interaction, $F(1,27)=4.23$, $p=0.04$; simple interaction effect of Trace, Superficial $F(1,27)=3.13$, $p=0.08$; Deep, $F(1,27)=0.02$, $p=0.87$). cA29c showed the highest levels of Zif268 than both cA29a and cA29b (Subregion main effect $F(2,54)=9.53$, $p<0.001$; simple main effects cA29c vs cA29a $F(1,27)=10.16$, $p=0.003$, cA29b $F(1,29)=18.17$, $p<0.002$), while cA29a and cA29b did not differ ($F(1,27)=0.07$, $p=0.78$).

ATN lesions drastically reduced cell counts in cA30 as well, but there was no Layer by Lesion interaction (Lesion main effect $F(1,27)=69.40$, $p<0.001$; Layer x Lesion interaction ($F(1,27)=0.03$, $p=0.85$). The greatest reduction following ATN lesions was evident in the superficial layer (Layer x Lesion interaction, $F(1,27)=36.39$, $p<0.001$; simple interaction effect of Lesion: Superficial, $F(1,27)=64.22$, $p<0.001$, Deep, $F(1,27)=35.99$, $p<0.001$). Rats expressed higher counts of Zif268 when there was a Trace (Trace main effect, $F(1,27)=8.62$, $p=0.006$). There were no further interactions in cA30 (Trace by Layer, $F(1,27)=1.18$, $p=0.28$; Layer x Trace x Lesion interaction, $F(1,27)=0.71$, $p=0.40$).

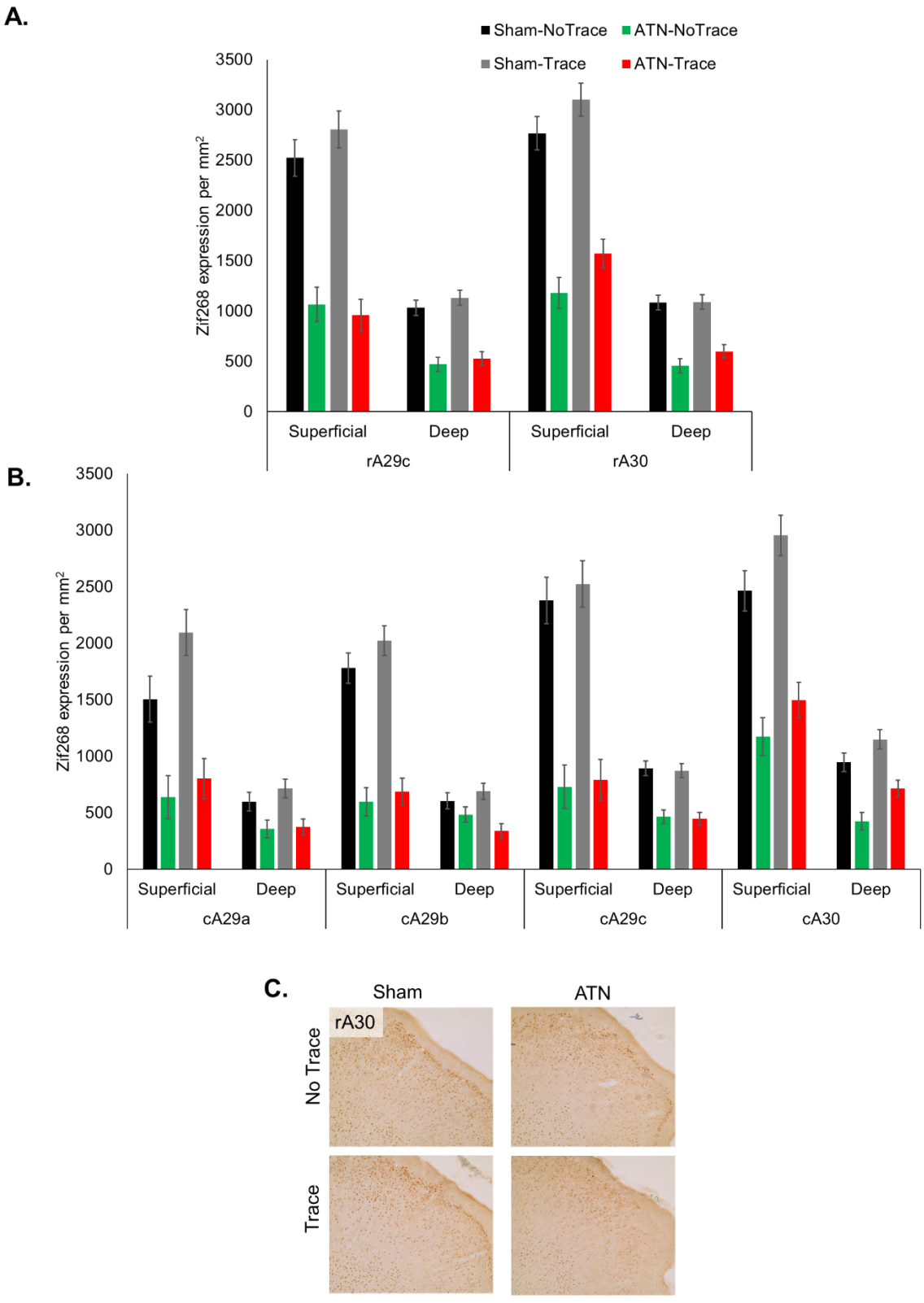

Figure 7.11. Zif268 expression per mm^2 in superficial and deep layers of both rostral (**A.**) and caudal (**B.**) retrosplenial cortex regions (Mean ± Std. Err). **C.** 10x magnification photomicrograph examples of Zif268 expression in rA30, representing median in each group. cA29a/b/c = caudal area 29a/b/c of the retrosplenial cortex; cA30 = caudal area 30 of the retrosplenial cortex; rA29c = rostral area 29c of the retrosplenial cortex; rA30 = rostral area 30 of the retrosplenial cortex.

Parahippocampal region

Zif268 expression for the perirhinal and entorhinal regions is shown in Figure 7.12. However, ATN lesions significantly reduced expression (Lesion main effect $F(1,27)=6.69$, $p=0.01$). There was no Trace by Lesion interaction ($F(1,27)=0.07$, $p=0.78$). There was no main effect of Trace on Zif268 expression in the perirhinal cortex (Trace, $F(1,27)=2.68$, $p=0.11$).

Although Sham No Trace rats showed higher expression in both the medial and lateral entorhinal cortex, there were no main effects or interactions seen in these regions (main effect of Trace $F(1,27)=0.17$, $p=0.67$; Lesion $F(1,27)=1.03$, $p=0.31$; Trace x Lesion interaction $F(1,27)=1.32$, $p=0.26$; Subregion x Trace x Lesion interaction $F(1,27)=0.41$, $p=0.52$).

No main effects or interactions of Trace and Lesion were found in the auditory control cortex (main effect: Trace, $F(1,27)=1.41$, $p=0.24$; Lesion, $F(1,27)=0.03$, $p=0.86$; Trace x Lesion interaction $F(1,27)=2.21$, $p=0.14$).

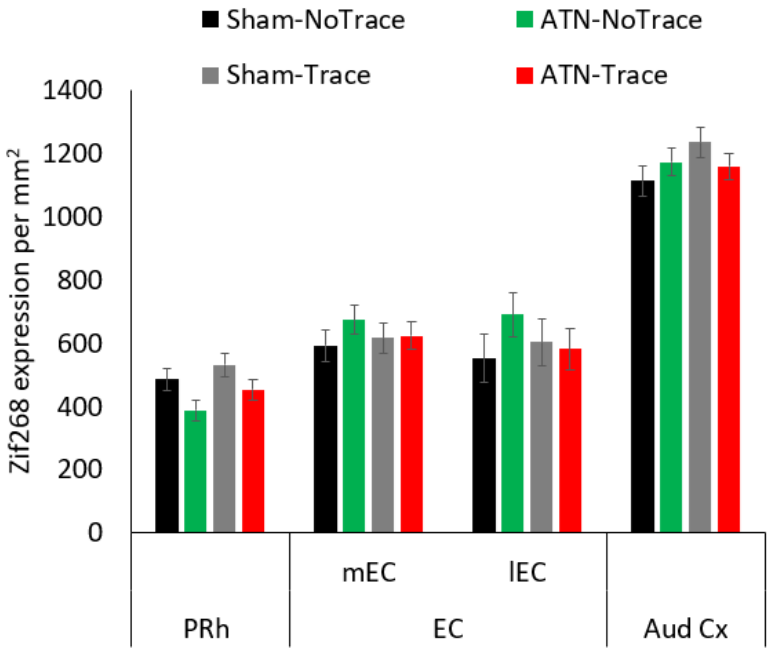

Figure 7.12. Zif268 expression per mm^2 in the parahippocampal cortex, including the perirhinal and entorhinal cortices, and the auditory control cortex (Mean ± Std. Err). lEC = lateral entorhinal cortex; mEC = medial entorhinal cortex; PRh = perirhinal cortex.

Comparison of hippocampal expression across intact/sham rats (Chapters 6 and 7)

A comparison of Zif268 dorsal hippocampal expression between the two temporal experiments (trace and non-trace; Chapter 6 and 7) is shown in Figure 7.13. There is considerably higher counts of Zif268 expression in the CA1 subregion ($F(3,81)=1860.62$, $p<0.001$). Rats in both experiments expressed higher counts of dorsal CA1 and CA3 Zif268 when there was a Trace (Trace main effect, $F(1,27)=10.56$, $p=0.003$; Subregion x Trace $F(3,81)=8.71$, $p<0.001$; simple interaction effect of Trace in: dCA1 ($F(1,27)=12.16$, $p=0.001$; dCA3, $F(1,27)=11.57$, $p=0.002$; DG, $F(1,27)=1.40$, $p=0.24$; DGhilus, $F(1,27)=0.21$,$p=0.64$). There was no main effect of Experiment or Trace by Experiment interaction in the dorsal HPC (Experiment main effect, $F(1,27)=0.41$, $p=0.52$; Trace by Experiment interaction ($F(1,27)=0.39$, $p=0.53$).

Rats in the current experiment (Expt. 3) expressed higher counts of Zif268 in the CA1 and cA3 subregions than rats in Expt. 2 (Chapter 6; Subregion x Experiment interaction, $F(3,81)=14.82$, $p<0.001$; simple interaction of Experiment in: dCA1, $F(1,27)=7.06$, $p=0.01$; CA3, $F(1,27)=8.41$, $p=0.007$). Conversely, rats in Expt. 2 expressed significantly higher counts the Expt. 3 in the DG, with no difference in expression in the DGhilus (simple interaction of Experiment in: DG, $F(1,27)=44.40$, $p<0.001$; DGhilus, $F(1,27)=1.36$, $p=0.25$). There was no Trace by Experiment by Subregion interaction ($F(3,81)=0.96$, $p=0.41$).

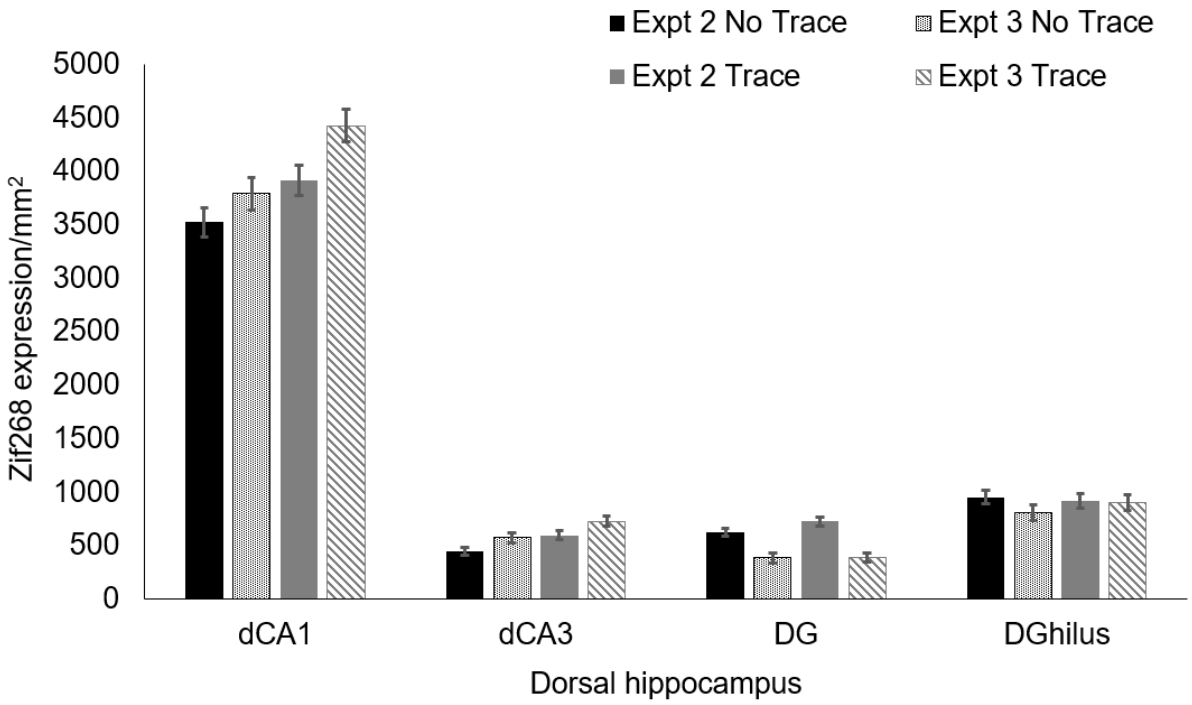

Figure 7.13. Comparison of Zif268 expression across intact (Expt 2) and sham rats (Expt 3, current study) in the dorsal hippocampus. Mean (± Std Err) Zif268 expression across the dorsal hippocampus. dCA1 = Cornu Ammonis Area 1 of the dorsal hippocampus; dCA3 = Cornu Ammonis Area 3 of the dorsal hippocampus; DG= dentate gyrus of the dorsal hippocampus; DGhilus = hilus of the dentate gyrus of the dorsal hippocampus; No Trace = odour-object paired associate task; Trace = odour-trace-object paired-associate task.

7.4 Discussion

The present study provides the first evidence that ATN lesions in rats produce deficits in the acquisition of a non-spatial paired-associate task. This deficit occurred irrespective of whether a temporal component was included. The ATN are important in human memory as injury or

dysfunction in the ATN is associated with severe memory disorders, including the alcoholic Korsakoff's syndrome, thalamic stroke, and early Alzheimer's disease (Aggleton et al., 2016; Carlesimo et al., 2011; Child & Benarroch, 2013; Kopelman, 2015). Contributions of additional damage cannot be ruled out in human studies, so experimental lesion studies such as that used here reinforce the specific role of the ATN in memory. As discussed in Chapter 4, extensive evidence shows that ATN lesions impair the ability to recall previously visited locations, such as in the radial-arm maze. In the current experiment, confirmed severe deficits on a spatial working memory task following ATN lesions. The current study adds a significantly important deficit. Paired-associate learning provides a different measure of episodic-like memory, especially when non-spatial non-temporal events are used. It is clear, at least in rats, that this non-spatial memory is also severely impaired by ATN lesions. Hence the effects of ATN lesions are clearly not restricted to their impact on spatial memory or temporal (recency) memory (Dumont & Aggleton, 2013; Frost et al., 2020; Wolff et al., 2006).

The inability to process spatial memory following injury to the ATN, suggests that the deficits from paired-associate spatial tasks cannot be confidently attributed to an impairment specific to the formation of the arbitrary association (Gibb et al., 2006; Henry et al., 2004; Sziklas & Petrides, 1999). The conclusion of impairments following ATN lesions when there is no spatial requirement is therefore an extremely important extension to the deficits previously described after ATN lesions. Before accepting this conclusion, comment of the task procedures is warranted. The apparatus was contained in black curtains with no direct lighting above, thereby removing any confound due to distal cues that may have helped the intact rats. The odours and objects within the apparatus, and the position of the experimenter outside of black curtains, were always the same for every trial, irrespective of task or trial. These procedures meant that it was extremely unlikely that rats would be able to employ spatial strategies to solve the task. Moreover, rats with ATN lesions were able to acquire the

simple odour and object discriminations as rapidly as controls. Thus, a fundamental deficit in response inhibition or response speed cannot explain the deficit found in the paired-associate tasks.

While the prediction that ATN lesions would cause a deficit in the odour-trace-object paired-associate task was confirmed, the contrary prediction, that a non-trace version would be only mildly affected by these lesions was not supported. Deficits following ATN lesions were clear in both No Trace and Trace variations of this task. It is possible, however, that the odour-object (No Trace) task contained a minor temporal element, because the two stimuli were not presented at the same time, with a ~1 second delay between presentations of stimuli. The odour was presented allowing the rat to consume the food reward before the object was exposed by removing the door. A different object-odour version of a paired-associate task was used by Gilbert and Kesner (2002). Their earlier task presented both the object and odour stimuli contiguously and found no behavioural deficits following HPC lesions. It remains possible that the introduction of even a minor temporal component introduces hippocampal temporal-like deficits in these rats. This explanation seems unlikely, however, to have produced the severity of deficits being equal across the two paired-associate task versions. Support that the 'no trace' and 'trace' (10 second delay) versions are not identical comes from evidence of a clear interaction with lesion status for Zif268 expression in CA1 neurons.

Distal hypoactivation throughout the 'hippocampal-diencephalic-cingulate' memory network (Bubb et al., 2017) shows the impact that the ATN lesions had on a wide range of cortical and hippocampal sites. Previous evidence shows that ATN lesions substantially reduce dendritic spine density in the CA1, despite no direct neural connections between these two regions (Harland et al., 2014). This outcome has been replicated, albeit after MTT lesions that would exert their impact through dysfunction of ATN neurons (Dillingham et al., 2019).

The impact of ATN lesions on the CA1 may have been sufficient to remove the ability to form the arbitrary associations (Kesner et al., 2005). The impact of ATN lesions on the no-trace (non-temporal) version of the task does was perhaps not related to Zif268 expression, however, as the level of Zif268 expression in the CA1 was similar in the ATN and Sham groups on that condition. ATN lesions do impact regions beyond the hippocampus. For example, Frost et al. (2020), although examining spatial coding, found evidence that ATN lesions significantly disrupt subiculum place cell units while leaving CA1 place cell units intact. This is indicative of the extensive direct and indirect connectivity of the ATN with regions within the 'hippocampal-diencephalic-cingulate' network (Bubb et al., 2017). Perhaps more related to the current non-spatial task, the current study also found clear evidence of hypoactivation throughout the medial prefrontal cortex, cingulate cortex and retrosplenial cortex as well as smaller changes in the perirhinal cortex. Severe behavioural deficits seen in this experiment may therefore be due to disruption at multiple points across the extended system.

Rats responded differently to the two Trace conditions. Rats in both lesion groups responded faster to correct pairings in the odour-trace-object task than did both groups to correct pairings in the odour-object (non-trace) task. This is of particular interest as ATN-lesion rats, while unable to acquire the paired-associate task, still altered responding to the temporal addition, although it is unclear why. Further investigation into the potential involvement of the ATN in temporal processing is required.

The ability to acquire two simple discriminations (odour and object), presented prior to paired-associate training, suggests that recovery over time cannot explain the difference between simple discrimination learning and performance on the paired-associate tasks. The ability to acquire simple discriminations indicates that any deficits in found in the paired-

associate tasks cannot be due to the inability to discriminate between the individual components. As mentioned above, ATN-lesion rats were able to inhibit responding in both the simple discrimination tasks. Moreover, latency speeds for both ‘go’ and ‘no-go’ trials indicate that the ATN-lesion rats were not running any faster than Sham-lesion animals. Similarities in latency suggest that there was no impact of lesion on attention or interaction with the objects. This reinforces the conclusion that the severe behavioural deficits were due to mnemonic dysfunction following ATN lesions, rather than to do with poor attention to task stimuli or lack of inhibitory control.

A potential limitation to this experiment was that human interaction was required to run the task. Although care was taken to ensure consistency in the handling of rats and the apparatus across trials, there is a chance subtle clues may have contributed behavioural performance. The experimental procedures were kept as consistent as possible, with all stimulus preparations taking place away from the apparatus. For example, ‘go’ trial food rewards were placed underneath the objects prior to securing them in place within the apparatus. This ensured that the rat did not get any additional odour or visual cues prior to the trial as to whether the object would contain a reward.

Reward value and possible reward devaluation may have influenced motivation in ATN-lesion rats to interact with objects. All trials within a session provided the rats with at least one chocolate reward in the odour receptacle. This was to ensure that each rat was interacting with the odour. One potential limitation of this is that it may have diminished the reward salience of the object reward on correct paired trials. All rats were placed on a restricted diet and trained prior to feeding to ensure high levels of motivation for responding for food rewards. ATN lesions, however, have not been shown to be involved in reward devaluation (Alcaraz et al., 2014). Consistent with this, latency to respond to correct pair trials

in ATN-lesion rats was comparable to controls. Salience of the cues used in this task may have also influenced responding. The cues used were visually different in terms of colour tone (black and white) and in height (tall and short). Correct pairings were also counterbalanced across the rats and all three studies (Chapters 5, 6, and 7). The height of the objects cannot have had an impact on acquisition as the rats were able to discriminate objects based on height in the simple object discrimination task. In this experiment, rats had to interact with each object and odour, ensuring that attention was given to all stimuli in order to gain a reward. A variation of objects could be used in the future, i.e. different textures. Conversely, this may lead to more variability in responding due to altered salience levels of vastly different objects.

I conclude that the ATN plays a critical role in supporting the acquisition of non-spatial arbitrary associations. This evidence is reinforced by the similar behavioural acquisition of both intact rats (Chapter 6) and Sham-lesion rats of the current study, which emphasises the poor acquisition shown by rats with ATN lesions. Due to the inability to acquire either paired-associate task following ATN injury, effects of these lesions on consolidation could not be tested (as that requires a relevant task in which no acquisition impairment is found). This study also replicated the increase in Zif268 expression in dCA1 in intact rats when a trace component is added to the odour-object paired-associate task. These findings suggest that the interdependency of the ATN and HPC is especially relevant in the acquisition of both temporal and non-temporal odour-object paired-associate tasks, but it also suggests that the impact of the ATN on multiple regions in the extended memory circuit may collectively explain the severe acquisition impairment observed in the current study.

Chapter 8.
General Discussion

8.1 Summary of findings

Significant memory deficits follow injury or dysfunction in regions within the 'hippocampal-diencephalic-cingulate' memory network (Bubb et al., 2017). This thesis summarised both clinical and experimental research on diencephalic amnesia, with a focus on the ATN. The ATN have dense reciprocal direct and indirect connections throughout 'hippocampal-diencephalic-cingulate' memory network and provide a neural hub within this complex network. It is well-established that injury to the ATN in animals reliably produces severe spatial memory deficits and decrease functional markers in distal structures in the wider system, most consistently in the retrosplenial cortex. The current study confirmed that ATN lesions produce marked impairments in spatial working memory. It then demonstrated important novel findings to add to the limited evidence of ATN involvement beyond spatial memory. Specifically, ATN lesions prevented the acquisition of non-spatial odour-object paired-associate tasks, irrespective of whether the task included an explicit temporal component. Deficits in the non-temporal version of the task suggest that the ability to form an arbitrary representation per se is a feature of ATN injury that suggests a profound impact on episodic memory. This has been suspected previously, but not empirically demonstrated. After a recall test 5 days after the end of acquisition, rats with ATN lesions also showed reduced Zif268 markers of neuronal activation across many structures in the memory system. This included the loss of increased neuronal activity in dorsal CA1 neurons exhibited by Sham rats when specifically trained on the temporal variant of the odour-object paired associate task. Nonetheless, the influence of the ATN clearly extends beyond spatial and temporal aspects of memory.

Before examining the effects of ATN lesions, the first two studies in the current thesis assessed task-specific activation in intact rats following retention of this novel non-spatial odour-object paired-associate task. This work began by identifying patterns of neural activation involved in consolidation of a non-spatial odour-trace-object association task (Chapter 5). This step was essential to determine that intact rats were able to acquire the odour-trace-object paired-association task, and to identify the pattern of neural activation followed both recent (5-day) and remote (25-day) recall of this non-spatial task. The superficial medial prefrontal cortex was identified as a critical region for remote recall of this non-spatial temporal task. We then identified patterns of neural activation in both a temporal and non-temporal non-spatial odour-object task when examined at 5-days post-acquisition (Chapter 6). This study compared acquisition of the previous odour-trace-object task to a non-temporal odour-object paired-associate task. We identified that the dorsal CA1 showed increased neural activation for recall of the odour-object paired-associate task when a temporal lag was introduced between presentations of the stimuli. The latter finding was replicated in the Sham group in the ATN lesion study. This work provides convergent evidence, in addition to the CA1 lesion work by Kesner et al. (2005), for a role of CA1 neurons in processing memory-relevant temporal information.

Using evidence from the first two studies, combined with the expectation that ATN and hippocampal injury produce interdependent effects, it was predicted (Chapter 7) that ATN lesions would produce a dissociation in the non-spatial paired-associate task. That is, deficits after ATN lesions were anticipated in the odour-trace-object task but not the non-trace version of this task. Of specific relevance, ATN lesions have previously been reported to produce microstructural changes in the dorsal CA1 region (Harland et al., 2014), which has been replicated after MTT lesions (Dillingham et al, 2019). It was, therefore, reasonable to predict

that injury to the ATN may produce deficits similar to those of the dorsal CA1 in the object-trace-odour task (Kesner et al., 2005). Contrary to the predicted dissociation, this study identified severe deficits after ATN lesions in the ability to form an association between the odour and object, irrespective of the presence of an explicit temporal lag between the stimuli.

This General Discussion addresses the empirical work and their theoretical value, followed by consideration of the limitations of the current study as well as suggestions regarding future directions.

8.2 Neural response the non-spatial odour-object paired-associate task

To identify the regions within the 'hippocampal-diencephalic-cingulate' network (Bubb et al., 2017) involved in retention of a non-spatial odour-trace-object, I assessed Zif268 expression at both 5- and 25-day recall (Chapter 5). In order to control for non-mnemonic IEG activation following the extended consolidation period, the third group ('new learning') was exposed to a novel pairing at 25-days (remote) on the original odour-trace-object procedure. Zif268 was used as to identify patterns of neural activation following retention of the previously acquired memory or exposure to novel stimuli (new learning). This study demonstrated an increase in activation of the superficial prefrontal cortex following remote recall compared to both recent recall and new learning of the odour-trace-object task. No apparent effects of remote recall on this paired-associate task were found in other neural structures. This activation pattern relative to both recent recall and new learning suggests that activation of medial prefrontal cortex was not simply related to the level of memory performance, but reflected specific consolidation of this non-spatial odour-trace-object task. The use of the "new learning" group means that the interval between testing, per se, could also not explain the difference between the T25 and T5 groups.

This study provides further supporting evidence for the recruitment of prefrontal cortical structures in long-term retention (consolidation) of memory. This is consistent with previous evidence of prefrontal cortical recruitment in mice following long-term retention of a spatial memory task (Bontempi et al., 1999; Maviel et al., 2004). Evidence from these studies demonstrate an increase in both immediate early gene and metabolic markers in the prefrontal cortex at 30-day recall compared to recent recall 1-day following task acquisition. This previous work did not identify cell-layer distribution in the prefrontal cortex, however, so we cannot directly compare the previous spatial memory task with the current evidence of superficial upregulation of Zif268 in the medial prefrontal cortex for a non-spatial paired-associate memory.

Previous work by Maviel et al. (2004) also demonstrated a time-depending shift in laminar organisation of the lateral parietal association cortex in their spatial task. They uncovered the time-dependent shift in laminar organisation from deep cortical layers (Layers V and VI) to superficial layers (Layers II, III, and IV) of the parietal cortex's neuronal activity during consolidation of remote memory. It was concluded that this region may play an integral role in integration of the spatial memory for permanent storage. There was no evidence of laminar reorganisation in the parietal cortex of intact rats in the current study's non-spatial task. The difference in parietal expression may be due to the non-spatial or temporal components of the current task, and of course, the different species used to test time-dependent neural activity. A direct comparison between these tasks, in one species, may resolve this issue.

There was, unexpectedly, no difference in hippocampal activation between recent and remote recall in the current study. This contrasts with previous evidence of downregulation of hippocampal Zif268 in consolidation of a spatial memory task and the broader expectation

that the hippocampus's role in episodic-like memory would diminish over time (Maviel et al., 2004). Kesner et al. (2005) established that hippocampal CA1 integrity is essential for temporal paired-associate acquisition in their object-trace-odour task. The current study is the first study to determine whether remote recall of a temporal paired-associated may differ from recent recall of that same temporal task. Even the 'new learning' group showed equivalent CA1 activation to those of the recall groups. Coupled with relatively lower activation across two studies when no trace was used in intact rats (Chapter 6 and Chapter 7), this suggests that CA1 activation is a key event during the non-spatial trace task, at least once rats have become familiar to the task procedures. That is, hippocampal involvement may be obligatory, irrespective of the novelty of the task or the time since acquisition. By contrast, other brain regions appeared to be involved in recall of the odour-trace-object paired-associate memory task, but not current processing of trace information once the task had been acquired. The most prominent region in this regard was the retrosplenial cortex. Intact rats in the experiments in Chapters 6 and 7 showed elevated retrosplenial cortex Zif268 expression in the trace version of the task compared to the non-trace version, especially in the superficial layers. This also provides additional evidence of retrosplenial cortex involvement in the recall of a task with temporal information. Previous evidence for the retrosplenial cortex in temporal memory has used recency/temporal order memory (Hayashi, Oguro, & Sato, 2020; Powell et al., 2017). Lesions of the retrosplenial cortex, in both temporal and non-temporal variants of the task, would indicate whether this cortex plays a more general role in paired-associate memory. While there is evidence that the retrosplenial cortex plays a role in consolidation of object memory (Magdalena, Medina, & Cynthia, 2020), the current study failed to find evidence from intact rats that the retrosplenial cortex is preferentially activated by remote as opposed to recent recall of a paired-associate memory representation.

The second experiment assessed the regional expression of Zif268 5-days (recent) post-acquisition to compare neural activation patterns specific to recall of an odour-object (non-trace) relative to an odour-trace-object paired associate task (Chapter 6). As predicted, intact rats were able to acquire the no-trace task at a similar rate to rats trained on the odour-trace-object task. This is the first study, to our knowledge, that examined patterns of neural activation in intact rats on recent recall in both a trace and non-trace variation of a non-spatial paired-associate task. The benefit of examining neural activation is that it describes potential active roles for individual neurons, including subregional and layer-specific neurons, in a given task.

This second study demonstrated evidence of higher hippocampal CA1 activity in the odour-trace-object relative to the odour-object paired associate task in the absence of an explicit inter-stimulus delay. This extends previous research, that rats with CA1 lesions, unlike those with CA3 lesions, are unable to acquire a non-spatial paired-associate task when a delay is used (Kesner et al., 2005). At least when making an arbitrary association between an object and odour across a temporal lag, dorsal CA1 neurons appear to play a significant role. The increase of Zif268 expression found in the dorsal CA3 in the current study, however, was not expected. This finding suggests that, although acquisition is spared following dorsal CA3 lesions, dorsal CA3 neurons are also recruited by an intact hippocampus for recall of the trace paired-associate task. A small increase in CA3 activation was also found in the Sham-lesion rats in the third (ATN lesion) study, although the inclusion of ATN-lesion rats perhaps prevented a statistically reliable effect in those intact rats. A comparison of the dorsal HPC across intact (Chapter 6) and Sham lesion (Chapter 7), revealed the same pattern of activation in both the dorsal CA1 and CA3. Sham rats revealed a similar increase in Zif268 in these critical HPC regions following retention on the odour-trace-object task as intact rats from the previous cohort.

As mentioned above, the superficial layer of the rostral dysgranular retrosplenial cortex (A30) also showed upregulation following the recall of the odour-trace-object paired-associate task. This pattern of activation, although not significant, was also seen in the granular retrosplenial cortex. Despite being commonly implicated in spatial learning and memory (Aggleton & Pearce, 2001; Wyss & Van Groen, 1992), this pattern of activation implies that the superficial layers of retrosplenial cortex regions may also be recruited in this non-spatial temporal paired-associate task. Dense connectivity between the hippocampus and retrosplenial cortex (van Groen & Wyss, 1990b, 1992, 2003) may provide an explanation for the increase in activation following recall on the seemingly hippocampal-dependent 'trace' paired-associate task.

Taken together, patterns of activation demonstrated in the first two experiments (Chapters 5 and 6) indicate that the dorsal hippocampus is critical in recall of the odour-trace-object task and that there was a time-dependent shift to superficial prefrontal cortex recruitment in consolidation of this 'trace' paired-associate task. With other evidence in the literature, described above, the findings from these two experiments in intact rats added to the prediction that ATN lesions would produce acquisition deficits in the odour-trace-object task, specifically, and not in the non-trace version of the task. If so, the latter would provide a potential option (odour-object, without trace) to examine the neurobiological correlates of long-term consolidation after ATN lesions. Consolidation studies of ATN lesion effects are hampered by their use of spatial tasks and the acquisition deficits produced by ATN lesions (Lopez et al., 2009).

8.3 ATN lesions: Non-spatial odour-object and odour-trace-object deficits and lesion impact on the extended memory system

The final study demonstrated the first evidence that ATN lesions impair acquisition of a non-spatial paired-associate task in both a temporal and non-temporal context. As discussed in Chapter 4, extensive evidence shows that ATN lesions impair the ability to recall spatial memory. As expected, severe deficits were evident on a spatial memory RAM task following ATN lesions in the current study. Adding a significantly important deficit, beyond spatial memory, paired-associate learning (i.e. episodic-like memory) for non-spatial non-temporal representation was shown to also be severely impaired by ATN lesions. That is, contrary to predictions, deficits in acquisition following ATN lesions were severe in both temporal and non-temporal variations of this task. This demonstrates that ATN lesion deficits on a non-spatial task are not restricted to the inclusion of temporal processing (i.e. recency) or spatial information as previous evidence would suggest (Dumont & Aggleton, 2013; Frost et al., 2020; Wolff et al., 2006). There was no indication of recovery in the ATN lesion rats and no sign of learning the task even over extensive training sessions. This demonstrates the critical role of the ATN, at least in this context, for the acquisition of arbitrary associations of odours and objects irrespective of temporal presentation.

In addition to clear lesion deficits in the paired-associate tasks, rats responded differently to the two trace conditions. As mentioned in Chapter 7, this is of particular interest as ATN lesion rats, while unable to acquire the paired-associate task, still altered responding to the temporal addition. Further investigation into the potential involvement of the ATN in temporal processing is required.

As discussed in Chapter 7, it seems possible that the introduction of a minor temporal component (~1 second) may produce hippocampal temporal-like deficits in these rats.

However, we found a clear interaction with lesion status for CA1 Zif268 expression between the odour-object and odour-trace-object tasks, reinforcing that these tasks differ at least in terms of hippocampal recruitment. The impact of ATN lesions on the CA1 activation may have been sufficient to remove the ability to form the non-spatial arbitrary associations even with a minimal temporal component. Impairment associated with temporal memory subserved by the dorsal CA1 is supported by evidence that ATN lesions, as stated above, reduce the microstructural integrity of dorsal CA1 spines (Harland et al., 2014). Hippocampal Zif268 hypoactivation in ATN lesion rats from the current study is comparable to previous evidence of reductions following a novel cue radial arm maze task (Jenkins, Dias, Amin, Brown, et al., 2002; Perry et al., 2018). There is however, conflicting evidence of dorsal CA1 sparing following ATN lesions in c-Fos. For example Dumont et al. (2012) found no evidence of lesion effects in the dorsal hippocampus 90 minutes after the final spatial memory testing session.

Severe behavioural deficits on the non-spatial paired-associate memory tasks may be due to ATN lesions disrupting a crucial node in the interconnected system of structures related to episodic memory. There is consistent evidence of system-wide alterations of IEGs as a result of ATN lesions (discussed in Chapter 4). The current study also found evidence of hypoactivation throughout the medial prefrontal, cingulate (ACC and RSC) and perirhinal cortices. A number of previous studies have also found decreased expression in c-Fos and Zif268 comparable to the current study in the ACC (Dupire et al., 2013; Jenkins, Dias, Amin, Brown, et al., 2002; Perry et al., 2018) and especially RSC (Dumont et al., 2012; Jenkins, Dias, Amin, Brown, et al., 2002; Loukavenko et al., 2015; Perry et al., 2018; Poirier & Aggleton, 2009). Notably, these studies focussed mostly on neural activation of the RSC following ATN lesions.

Paired-associate episodic-like tasks provide a measure of the unique representation of multiple stimuli following specific conditional rules, rather than the processing of an individual component of the task (Kehoe, 1988). Previous evidence from Kesner and colleagues demonstrated that hippocampal subregions implicated in odour-place, object-place and object-trace-odour tasks are responsible for impaired association memory for arbitrary stimuli due to the temporal and spatial components of these tasks (Gilbert & Kesner, 2002; Kesner et al., 2005). Lesion studies provide evidence of deficits following the removal of a structure, but do not directly demonstrate that the region plays an active, critical role in the specific task. The current study drew direct comparisons across the non-spatial odour-object and odour-trace-object tasks in intact rats, providing evidence of neural activation in key regions implicated in both 'trace' conditions of the paired-associate task. This novel approach is important to determine how key structures, such as the hippocampus and prefrontal cortex, respond to consolidation and temporal variations following acquisition on the task. The replicated involvement of the dorsal CA1 and CA3, shown by increased activation following recall in both intact (Chapters 6) and Sham lesion rats (Chapter 7) points to their direct involvement in memory processing for the odour-trace-object task.

It is possible that severe deficits in both temporal and non-temporal variations of the odour-object paired-associate task was due to the sequential presentation of the stimuli (Manns & Eichenbaum, 2005). That is, that the presentation of stimuli at separate times within a trial may rely on intact functioning of the ATN and its connectivity through the 'hippocampal-diencephalic-cingulate' network (Bubb et al., 2017). A deficit in processing of sequential stimuli would be consistent with previous work showing behavioural deficits following ATN lesions in the processing the temporal order of odours (Wolff et al., 2006). Other studies have also shown that the hippocampus is required for memory of the sequence of events (Fortin et al., 2002; Kesner et al., 2002). This is the first study to measure

discrimination of pairings presented in this fashion (odour presented first, then object) with and without a temporal context. The ATN therefore, may be integral in the processing of these sequential stimuli. CA1 lesions did not impair acquisition of a non-temporal object-odour task when stimuli were presented contiguously on a cheeseboard (Gilbert & Kesner, 2002). In order to determine whether the ATN impaired the current non-temporal task due to sequential presentation of stimuli, performance on a similar cheeseboard variation of our non-spatial odour-object paired-associate task could be investigated.

The results of this study strongly indicate that the ATN has a critical role in the extended memory system with a strong influence on memory beyond 'space'.

8.4 Limitations of the current study

The main limitation of the current study was the time-consuming nature of the behavioural task used. Both odour-object and odour-trace-object tasks required 12 massed trials per rat, per day, restricting the number of rats that could be trained on the task. Nonetheless, specific task differences found were found between groups in each experiment. In the first experiment (Chapter 5), active controls were only assigned to the remote recall group. There were no active controls on either temporal context when tested at recent recall, but performance was similar at recall for both groups indicating any variation in neural activation could be attributed to the different task demands.

As discussed in Chapter 5, one limitation of all three experiments is the lack of home cage controls to form 'baseline' Zif268 activation levels in all regions of interest. Such 'baseline' activation would help to identify regions critical to learning and memory through identification of non-specific neural activation patterns unrelated to learning and memory or exposure to novel stimuli. The dorsal hippocampus was identified as a critical region in

acquisition of the odour-trace-object paired associate task, but no active controls (a ‘new learning’ group) were tested at recent recall to determine whether neural activation was a bi-product of being put back in a familiar apparatus. ‘Baseline’ measures of non-specific neural activation (home caged controls) could provide valuable information regarding activation levels in the hippocampus, for example, relative to rats not exposed to any task-relevant stimuli, temporal or non-temporal.

The assessment of only one neural activation marker at only one time point post retention test limits the ability to make strong conclusions as to the link between behaviour and neural activation. Assessment of further IEGs, such as c-Fos, and other metabolic markers may identify further patterns of activation not shown by Zif268 in the current study. Measuring neural markers at one time point (90 minutes post-retention) limits conclusions that can be made based on activation levels. There is, for example, no indication of neural changes over time, both within and between memory related structures. Electrophysiological recording over time could produce insightful information regarding the time-dependent recruitment of diencephalic and cortical regions at different time points through training.

Another potential limitation was the possibility that the objects were not different enough for the complex nature of the discrimination task. Despite similar responding to both pairings, the task may have been easier for the rats to acquire had the two objects been more visually distinct from each other. This however, comes with its own downfalls, by using more varied stimuli the salience of one may be more so than the other, unintentionally skewing behavioural responding. Acquisition shown by Sham groups indicates that the height and colour difference of the objects was sufficient for learning the paired-associate tasks.

8.5 Future directions

One potential direction would be to adopt an 'active control' task that does not require forming an association between stimuli. Such a task may be a simple discrimination with both an odour and an object present. For example, a simple discrimination between two objects could be used, but with a single (non-varying) odour presented in the runway in every trial; the odour would be redundant and only the object discrimination remain relevant. This would provide an experience comparable in design to the odour-object task without the rat needing to acquire a paired-associate task. If acquired prior to assessment of neural activation then this modified task would provide insight into activation patterns associated with exposure to the stimuli, but not the temporal or non-temporal paired associate task. Combined with 'baseline' controls, as described above, clear insight into specific neural activation patterns specific to the paired-associate task could be determined.

It would be of considerable interest to determine the role of further diencephalic structures in the non-spatial odour-object and odour-trace-object paired-associate tasks. As discussed in Chapter 2, clinical amnesia has been associated with injury to many structures in the diencephalon. For example, lesions to the mammillothalamic tract or especially the mediodorsal thalamic nuclei (MD) could determine if this behavioural deficit is unique to the anterior thalamus, or if the severity of deficits (with and without the temporal component) differ between regions. Future work could also look at whether the effect seen in the current study differed if there was selective injury to one of the subnuclei of the ATN. Lesion studies to individual AM or AV/AD nuclei followed by training in both the temporal and non-temporal tasks could determine the role of these nuclei in a non-spatial odour-object paired-associate task. Early work in my PhD aimed to determine the comparative role of the ATN and MD on both spatial and associative learning and memory. Due to unavoidable circumstances this experiment was unfortunately unable to continue. Group numbers were too

small due to anaesthesia/surgical complications, compounded by the fact that female rats were used that seemed prone to pituitary tumours. Variable lesions in the small number of rats left, resulted in the inability to make firm conclusions of lesion effects on behaviour. It would be interesting to see a similar study done in male rats with contralateral and bilateral ATN and MD lesions in a non-spatial paired-associate task.

As mentioned in the limitations, further analysis of neural activation may supplement the current evidence of activation patterns. This could be done in both consolidation and temporal and non-temporal contexts of the odour-object paired associate task. One example would be to look at the effects of c-Fos in the same memory related structures in order to directly compare these two IEGs following retention of a temporal and non-temporal non-spatial paired-associate task. In the past, I have not had any success in visualising c-Fos with the antibodies that have been available in the lab. Further trials perhaps with antigen retrieval steps, or finding a new antibody source, may remedy this. Again, analysis of neural activation at different time-points of training with electrophysiological measures would provide invaluable insight into acquisition of this novel odour-object paired-associate task either with or without the temporal lag.

8.6 Summary

In summary, the amnesic syndrome associated with the ATN does not appear to be limited to spatial memory or even temporal memory. This thesis provides the first example of non-spatial paired-associate deficits following injury to the ATN. Contrary to expectations, ATN lesions produced severe impairments in both an odour-object (non-trace) and odour-trace-object paired-task. Moreover, extensive training demonstrated no recovery of learning in these ATN lesion rats, demonstrating the severity of these behavioural deficits. This study also demonstrated the first direct comparison of intact (and Sham lesion) rats in both a temporal

and non-temporal variation of an odour-object paired-associate task, and the resulting neural activation patterns. This provided critical insight into consolidation and recall of this non-spatial task, implicating the medial prefrontal cortex in consolidation and the hippocampus dorsal CA1 and CA3 in the odour-trace-object paired-associate task. Evidence from these paired-associate tasks confirms the interdependent nature of the ATN and hippocampus within the 'hippocampal-diencephalic-cingulate' memory network (Bubb et al., 2017). However, it also suggests that the impact of ATN lesions may reflect its impact across a variety of neural structures, at least when a non-spatial arbitrary representation is formed. This work provides robust additional support for the ATN as a critical node within the extended memory system supporting memory function and that it is unlikely that the ATN operates primarily as a relay for hippocampal information.

References

Aggleton, J. P. (2008). Understanding anterograde amnesia: Disconnections and hidden lesions. *QUARTERLY JOURNAL OF EXPERIMENTAL PSYCHOLOGY, 61*(10), 1441-1471. https://doi.org/10.1080/17470210802215335

Aggleton, J. P. (2012). Multiple anatomical systems embedded within the primate medial temporal lobe: implications for hippocampal function. *Neuroscience and biobehavioral reviews, 36*(7), 1579-1596. https://doi.org/10.1016/j.neubiorev.2011.09.005

Aggleton, J. P. (2014). Looking beyond the hippocampus: old and new neurological targets for understanding memory disorders. *Proceedings. Biological sciences / The Royal Society, 281*(1786), 20140565-20140565. https://doi.org/10.1098/rspb.2014.0565

Aggleton, J. P., Amin, E., Jenkins, T. A., Pearce, J. M., & Robinson, J. (2011). Lesions in the anterior thalamic nuclei of rats do not disrupt acquisition of stimulus sequence learning. *The Quarterly Journal of Experimental Psychology, 64*(1), 65-73.

Aggleton, J. P., & Brown, M. W. (1999). Episodic memory, amnesia, and the hippocampal-anterior thalamic axis. *Behavioral and Brain Sciences, 22*(3), 425-444. https://doi.org/10.1017/s0140525x99002034

Aggleton, J. P., & Brown, M. W. (2006). Interleaving brain systems for episodic and recognition memory. *Trends in Cognitive Sciences, 10*(10), 455-463. https://doi.org/10.1016/j.tics.2006.08.003

Aggleton, J. P., Hunt, P. R., Nagle, S., & Neave, N. (1996). The effects of selective lesions within the anterior thalamic nuclei on spatial memory in the rat. *Behavioural Brain Research, 81*(1), 189-198. https://doi.org/10.1016/S0166-4328(96)89080-2

Aggleton, J. P., & Nelson, A. J. D. (2015). Why do lesions in the rodent anterior thalamic nuclei cause such severe spatial deficits? *Neuroscience & Biobehavioral Reviews*.

Aggleton, J. P., & Nelson, A. J. D. (2020). Distributed interactive brain circuits for object-in-place memory: A place for time? *Brain and neuroscience advances, 4*, 2398212820933471.

Aggleton, J. P., O'Mara, S. M., Vann, S. D., Wright, N. F., Tsanov, M., & Erichsen, J. T. (2010). Hippocampal-anterior thalamic pathways for memory: uncovering a network of direct and indirect actions. *The European journal of neuroscience, 31*(12), 2292-2307. https://doi.org/10.1111/j.1460-9568.2010.07251.x

Aggleton, J. P., & Pearce, J. M. (2001). Neural systems underlying episodic memory: insights from animal research. *Philosophical Transactions of the Royal Society of London.Series B: Biological Sciences, 356*(1413), 1467-1482. https://doi.org/10.1098/rstb.2001.0946

Aggleton, J. P., Poirier, G. L., Aggleton, H. S., Vann, S. D., & Pearce, J. M. (2009). Lesions of the fornix and anterior thalamic nuclei dissociate different aspects of hippocampal-dependent spatial learning: implications for the neural basis of scene learning. *Behavioral Neuroscience, 123*(3), 504.

Aggleton, J. P., Pralus, A., Nelson, A. J. D., & Hornberger, M. (2016). Thalamic pathology and memory loss in early Alzheimer's disease: moving the focus from the medial temporal lobe to Papez circuit. *Brain, 139*(7), 1877-1890. https://doi.org/10.1093/brain/aww083

Albasser, M. M., Chapman, R. J., Amin, E., Iordanova, M. D., Vann, S. D., & Aggleton, J. P. (2010). New behavioral protocols to extend our knowledge of rodent object recognition memory. *Learning & memory (Cold Spring Harbor, N.Y.), 17*(8), 407-419. https://doi.org/10.1101/lm.1879610

Alberini, C. M. (2009). Transcription factors in long-term memory and synaptic plasticity. *Physiological reviews, 89*(1), 121-145.

Alcaraz, F., Naneix, F., Desfosses, E., Marchand, A. R., Wolff, M., & Coutureau, E. (2014). Dissociable effects of anterior and mediodorsal thalamic lesions on spatial goal-directed behavior. *Brain Structure and Function*, 1-11.

Alexinsky, T. (2001). Differential effect of thalamic and cortical lesions on memory systems in the rat. *Behavioural Brain Research, 122*(2), 175-191. https://doi.org/10.1016/S0166-4328(01)00182-6

Allen, G. V., & Hopkins, D. A. (1988). Mamillary body in the rat: a cytoarchitectonic, Golgi, and ultrastructural study. *Journal of Comparative Neurology, 275*(1), 39-64.

Barker, G. R. I., Bird, F., Alexander, V., & Warburton, E. C. (2007). Recognition memory for objects, place, and temporal order: a disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. *Journal of Neuroscience, 27*(11), 2948-2957.

Barker, G. R. I., & Warburton, E. C. (2008). NMDA receptor plasticity in the perirhinal and prefrontal cortices is crucial for the acquisition of long-term object-in-place associative memory. *Journal of Neuroscience, 28*(11), 2837-2844.

Barker, G. R. I., & Warburton, E. C. (2018). A critical role for the nucleus reuniens in long-term, but not short-term associative recognition memory formation. *Journal of Neuroscience, 38*(13), 3208-3217.

Barry, D. N., Coogan, A. N., & Commins, S. (2016). The time course of systems consolidation of spatial memory from recent to remote retention: A comparison of the Immediate Early Genes Zif268, c-Fos and Arc. *Neurobiology of Learning and Memory, 128*, 46-55.

Bentivoglio, M., Kultas-Ilinsky, K., & Ilinsky, I. (1993). Limbic thalamus: structure, intrinsic organization, and connections. In *Neurobiology of cingulate cortex and limbic thalamus* (pp. 71-122): Springer.

Binder, S., Dere, E., & Zlomuzica, A. (2015). A critical appraisal of the what-where-when episodic-like memory test in rodents: Achievements, caveats and future directions. *PROGRESS IN NEUROBIOLOGY, 130*, 71-85.

Bontempi, B., Laurent-Demir, C., Destrade, C., & Jaffard, R. (1999). Time-dependent reorganization of brain circuitry underlying long-term memory storage. *Nature, 400*(6745), 671-675.

Braak, H., & Braak, E. (1991a). Alzheimer's disease affects limbic nuclei of the thalamus. *Acta neuropathologica, 81*(3), 261-268.

Braak, H., & Braak, E. (1991b). Neuropathological stageing of Alzheimer-related changes. *Acta neuropathologica, 82*(4), 239-259.

Brown, M. W., & Aggleton, J. P. (2001). Recognition memory: what are the roles of the perirhinal cortex and hippocampus? *Nature Reviews Neuroscience, 2*(1), 51-61.

Bubb, E. J., Kinnavane, L., & Aggleton, J. P. (2017). Hippocampal–diencephalic–cingulate networks for memory and emotion: An anatomical guide. *Brain and neuroscience advances, 1*, 2398212817723443.

Bubb, E. J., Metzler-Baddeley, C., & Aggleton, J. P. (2018). The cingulum bundle: Anatomy, function, and dysfunction. *Neuroscience & Biobehavioral Reviews, 92*, 104-127.

Burwell, R. D. (2000). The parahippocampal region: corticocortical connectivity. *Annals-New York Academy of Sciences, 911*, 25-42.

Bussey, T. J., Saksida, L. M., & Murray, E. A. (2005). The perceptual-mnemonic/feature conjunction model of perirhinal cortex function. *The Quarterly Journal of Experimental Psychology Section B, 58*(3-4), 269-282.

Byatt, G., & Dalrymple-Alford, J. C. (1996). Both Anteromedial and Anteroventral Thalamic Lesions Impair Radial-Maze Learning in Rats. *Behavioral Neuroscience, 110*(6), 1335-1348. https://doi.org/10.1037/0735-7044.110.6.1335

Carlesimo, G. A., Lombardi, M. G., & Caltagirone, C. (2011). Vascular thalamic amnesia: A reappraisal. *Neuropsychologia, 49*(5), 777-789. https://doi.org/10.1016/j.neuropsychologia.2011.01.026

Carrera, E., & Bogousslavsky, J. (2006). The thalamus and behavior: Effects of anatomically distinct strokes. *Neurology*.

Carrera, E., Michel, P., & Bogousslavsky, J. (2004). Anteromedian, central, and posterolateral infarcts of the thalamus three variant types. *Stroke, 35*(12), 2826-2831.

Child, N. D., & Benarroch, E. E. (2013). Anterior nucleus of the thalamus: functional organization and clinical implications. *Neurology, 81*(21), 1869-1876. https://doi.org/10.1212/01.wnl.0000436078.95856.56

Cho, Y. H., & Kesner, R. P. (1995). Relational object association learning in rats with hippocampal lesions. *Behavioural Brain Research, 67*(1), 91-98.

Christiansen, K., Dillingham, C. M., Wright, N. F., Saunders, R. C., Vann, S. D., & Aggleton, J. P. (2016). Complementary subicular pathways to the anterior thalamic nuclei and mammillary bodies in the rat and macaque monkey brain. *European Journal of Neuroscience, 43*(8), 1044-1061.

Chudasama, Y., Bussey, T. J., & Muir, J. L. (2001). Effects of selective thalamic and prelimbic cortex lesions on two types of visual discrimination and reversal learning. *The European journal of neuroscience, 14*(6), 1009-1020.

Clayton, N. S., & Dickinson, A. (1998). Episodic-like memory during cache recovery by scrub jays. *Nature, 395*(6699), 272.

Cohen, N., J., & Eichenbaum, H. B. (1993). Memory, amnesia, and the hippocampal system.

Corbit, L. H., Muir, J. L., & Balleine, B. W. (2003). Lesions of mediodorsal thalamus and anterior thalamic nuclei produce dissociable effects on instrumental conditioning in rats. *European Journal of Neuroscience, 18*(5), 1286-1294. https://doi.org/10.1046/j.1460-9568.2003.02833.x

Cross, L., Brown, M. W., Aggleton, J. P., & Warburton, E. C. (2013). The medial dorsal thalamic nucleus and the medial prefrontal cortex of the rat function together to support associative recognition and recency but not item recognition. *Learning and Memory, 20*(1), 41-50. https://doi.org/10.1101/lm.028266.112

Dalrymple-Alford, J. C., Harland, B. C., Loukavenko, E. A., Perry, B., Mercer, S., Collings, D. A., . . . Wolff, M. (2015). Anterior thalamic nuclei lesions and recovery of function: Relevance to cognitive thalamus. *Neuroscience and Biobehavioural Reviews*.

Danet, L., Barbeau, E. J., Eustache, P., Planton, M., Raposo, N., Sibon, I., . . . Pariente, J. (2015). Thalamic amnesia after infarct: the role of the mammillothalamic tract and mediodorsal nucleus. *Neurology, 85*(24), 2107-2115.

Davis, S., Bozon, B., & Laroche, S. (2003). How necessary is the activation of the immediate early gene zif268 in synaptic plasticity and learning? *Behavioural Brain Research, 142*(1-2), 17-30.

de Jong, L. W., van der Hiele, K., Veer, I. M., Houwing, J. J., Westendorp, R. G. J., Bollen, E. L. E. M., . . . van der Grond, J. (2008). Strongly reduced volumes of putamen and thalamus in Alzheimer's disease: an MRI study. *Brain, 131*(12), 3277-3285.

Delacourte, A., David, J. P., Sergeant, N., Buee, L., Wattez, A., Vermersch, P., . . . Lebert, F. (1999). The biochemical pathway of neurofibrillary degeneration in aging and Alzheimer's disease. *Neurology, 52*(6), 1158-1158.

Delay, J., & Brion, S. (1969). *Le syndrome de Korsakoff*: Masson.

Delhaye, E., Bahri, M. A., Salmon, E., & Bastin, C. (2019). Impaired perceptual integration and memory for unitized representations are associated with perirhinal cortex atrophy in Alzheimer's disease. *Neurobiology of aging, 73*, 135-144.

Dillingham, C. M., Erichsen, J. T., O'Mara, S. M., Aggleton, J. P., & Vann, S. D. (2015). Fornical and nonfornical projections from the rat hippocampal formation to the anterior thalamic nuclei. *Hippocampus, 25*(9), 977-992.

Dillingham, C. M., Frizzati, A., Nelson, A. J. D., & Vann, S. D. (2015). How do mammillary body inputs contribute to anterior thalamic function? *Neuroscience & Biobehavioral Reviews, 54*, 108-119.

Dillingham, C. M., Milczarek, M. M., Perry, J. C., Frost, B. E., Parker, G. D., Assaf, Y., . . . Vann, S. D. (2019). Mammillothalamic Disconnection Alters Hippocampocortical Oscillatory Activity and Microstructure: Implications for Diencephalic Amnesia. *Journal of Neuroscience, 39*(34), 6696-6713.

Dudai, Y. (2004). The neurobiology of consolidations, or, how stable is the engram? *Annu. Rev. Psychol., 55*, 51-86.

Dumont, J. R., & Aggleton, J. P. (2013). Dissociation of Recognition and Recency Memory Judgments After Anterior Thalamic Nuclei Lesions in Rats. *Behavioral Neuroscience, 127*(3), 415-431. https://doi.org/10.1037/a0032750

Dumont, J. R., Amin, E., & Aggleton, J. P. (2014). Selective importance of the rat anterior thalamic nuclei for configural learning involving distal spatial cues. *The European journal of neuroscience, 39*(2), 241-256. https://doi.org/10.1111/ejn.12409

Dumont, J. R., Amin, E., Poirier, G. L., Albasser, M. M., & Aggleton, J. P. (2012). Anterior thalamic nuclei lesions in rats disrupt markers of neural plasticity in distal limbic brain regions. *Neuroscience, 224*, 81-101. https://doi.org/10.1016/j.neuroscience.2012.08.027

Dumont, J. R., Amin, E., Wright, N. F., Dillingham, C. M., & Aggleton, J. P. (2015). The impact of fornix lesions in rats on spatial learning tasks sensitive to anterior thalamic and hippocampal damage. *Behavioural Brain Research, 278*, 360-374.

Dumont, J. R., Petrides, M., & Sziklas, V. (2010). Fornix and retrosplenial contribution to a hippocampo-thalamic circuit underlying conditional learning. *Behavioural Brain Research, 209*(1), 13-20. https://doi.org/10.1016/j.bbr.2009.12.040

Dupire, A., Kant, P., Mons, N., Marchand, A. R., Coutureau, E., Dalrymple-Alford, J. C., & Wolff, M. (2013). A role for anterior thalamic nuclei in affective cognition: interaction with environmental conditions. *Hippocampus, 23*(5), 392-404. https://doi.org/10.1002/hipo.22098

Dusoir, H., Kapur, N., Byrnes, D. P., McKinstry, S., & Hoare, R. D. (1990). The role of diencephalic pathology in human memory disorder: evidence from a penetrating paranasal brain injury. *Brain, 113*(6), 1695-1706.

Eacott, M. J., & Gaffan, E. A. (2005). The roles of perirhinal cortex, postrhinal cortex, and the fornix in memory for objects, contexts, and events in the rat. *The Quarterly Journal of Experimental Psychology Section B, 58*(3-4), 202-217.

Eacott, M. J., & Norman, G. (2004). Integrated memory for object, place, and context in rats: a possible model of episodic-like memory? *Journal of Neuroscience, 24*(8), 1948-1953.

Eichenbaum, H. B. (2000). A cortical–hippocampal system for declarative memory. *Nature Reviews Neuroscience, 1*(1), 41.

Eichenbaum, H. B., & Fortin, N. J. (2009). The neurobiology of memory based predictions. *Philosophical Transactions of the Royal Society B: Biological Sciences, 364*(1521), 1183-1191.

Emre, M. (2003). Dementia associated with Parkinson's disease. *The Lancet Neurology, 2*(4), 229-237.

Fortin, N. J., Agster, K. L., & Eichenbaum, H. B. (2002). Critical role of the hippocampus in memory for sequences of events. *Nature Neuroscience, 5*(5), 458-462.

Frankland, P. W., & Bontempi, B. (2005). The organization of recent and remote memories. *Nature Reviews Neuroscience, 6*(2), 119-130.

Frizzati, A., Milczarek, M. M., Sengpiel, F., Thomas, K. L., Dillingham, C. M., & Vann, S. D. (2016). Comparable reduction in Zif268 levels and cytochrome oxidase activity in the retrosplenial cortex following mammillothalamic tract lesions. *Neuroscience, 330*, 39-49.

Frost, B. E., Cafalchio, M., Martin, S. K., Islam, M. N., Aggleton, J. P., & O'Mara, S. M. (2020). Spatial Coding in the Subiculum Requires Anterior Thalamic Inputs. *bioRxiv*.

Furtak, S. C., Wei, S. M., Agster, K. L., & Burwell, R. D. (2007). Functional neuroanatomy of the parahippocampal region in the rat: the perirhinal and postrhinal cortices. *Hippocampus, 17*(9), 709-722.

Ghika-Schmid, F., & Bogousslavsky, J. (2000). The acute behavioral syndrome of anterior thalamic infarction: a prospective study of 12 cases. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society, 48*(2), 220-227.

Gibb, S. J., Wolff, M., & Dalrymple-Alford, J. C. (2006). Odour–place paired-associate learning and limbic thalamus: Comparison of anterior, lateral and medial thalamic lesions. *Behavioural Brain Research, 172*(1), 155-168. https://doi.org/10.1016/j.bbr.2006.05.017

Gilbert, P. E., & Kesner, R. P. (2002). Role of rodent hippocampus in paired-associate learning involving associations between a stimulus and a spatial location. *Behavioral Neuroscience, 116*(1), 63.

Gilbert, P. E., & Kesner, R. P. (2003). Localization of function within the dorsal hippocampus: the role of the CA3 subregion in paired-associate learning. *Behavioral Neuroscience, 117*(6), 1385.

Gratwicke, J., Jahanshahi, M., & Foltynie, T. (2015). Parkinson's disease dementia: a neural networks perspective. *Brain, 138*(6), 1454-1476.

Guzowski, J. F., Setlow, B., Wagner, E. K., & McGaugh, J. L. (2001). Experience-dependent gene expression in the rat hippocampus after spatial learning: a comparison of the immediate-early genesArc, c-fos, and zif268. *Journal of Neuroscience, 21*(14), 5089-5098.

Hall, J., Thomas, K. L., & Everitt, B. J. (2001). Cellular imaging of zif268 expression in the hippocampus and amygdala during contextual and cued fear memory retrieval: selective activation of hippocampal CA1 neurons during the recall of contextual memories. *Journal of Neuroscience, 21*(6), 2186-2193.

Halliday, G. M. (2009). Thalamic changes in Parkinson's disease. *Parkinsonism & related disorders, 15*, S152-S155.

Harding, A., Halliday, G., Caine, D., & Kril, J. (2000). Degeneration of anterior thalamic nuclei differentiates alcoholics with amnesia. *Brain : a journal of neurology, 123 (Pt 1)*(1), 141-154. https://doi.org/10.1093/brain/123.1.141

Harland, B. C., Collings, D. A., McNaughton, N., Abraham, W. C., & Dalrymple-Alford, J. C. (2014). Anterior thalamic lesions reduce spine density in both hippocampal CA1 and retrosplenial cortex, but enrichment rescues CA1 spines only. *Hippocampus, 24*(10), 1232-1247. https://doi.org/10.1002/hipo.22309

Hayashi, T., Oguro, M., & Sato, N. (2020). Involvement of the retrosplenial cortex in the processing of the temporal aspect of episodic-like memory in rats. *Neuroscience Research, 154*, 52-55.

Henry, J., Petrides, M., St.-Laurent, M., & Sziklas, V. (2004). Spatial conditional associative learning: Effects of thalamo-hippocampal disconnection in rats. *Neuroreport, 15*(15), 2427-2431. https://doi.org/10.1097/00001756-200410250-00025

Hopkins, D. A. (2005). Neuroanatomy of head direction cell circuits. *Head Direction Cells and the Neural Mechanisms of Spatial Orientation. MIT Press, Cambridge, MA*, 17-44.

Houtchens, M. K., Benedict, R. H. B., Killiany, R., Sharma, J., Jaisani, Z., Singh, B., . . . Bakshi, R. (2007). Thalamic atrophy and cognition in multiple sclerosis. *Neurology, 69*(12), 1213-1223.

Hunsaker, M. R., Thorup, J. A., Welch, T., & Kesner, R. P. (2006). The role of CA3 and CA1 in the acquisition of an object-trace-place paired-associate task. *Behavioral Neuroscience, 120*(6), 1252.

Jacobson, R. R., Acker, C. F., & Lishman, W. A. (1990). Patterns of neuropsychological deficit in alcoholic Korsakoff's syndrome. *Psychological Medicine, 20*(2), 321-334.

Jacobson, S., & Marcus, E. M. (2011). *Neuroanatomy for the neuroscientist*. New York: Springer.

Jankowski, M. M., Ronnqvist, K. C., Tsanov, M., Vann, S. D., Wright, N. F., Erichsen, J. T., . . . O'Mara, S. M. (2013). The anterior thalamus provides a subcortical circuit supporting memory and spatial navigation. *Frontiers in systems neuroscience, 7*(Journal Article), 45. https://doi.org/10.3389/fnsys.2013.00045

Jenkins, T. A., Amin, E., Pearce, J. M., Brown, M. W., & Aggleton, J. P. (2004). Novel spatial arrangements of familiar visual stimuli promote activity in the rat hippocampal formation but not the parahippocampal cortices: a c-fos expression study. *Neuroscience, 124*(1), 43-52.

Jenkins, T. A., Dias, R., Amin, E., & Aggleton, J. P. (2002). Changes in Fos expression in the rat brain after unilateral lesions of the anterior thalamic nuclei. *European Journal of Neuroscience, 16*(8), 1425-1432.

Jenkins, T. A., Dias, R., Amin, E., Brown, M. W., & Aggleton, J. P. (2002). Fos imaging reveals that lesions of the anterior thalamic nuclei produce widespread limbic hypoactivity in rats. *Journal of Neuroscience, 22*(12), 5230-5238.

Jenkins, T. A., Vann, S. D., Amin, E., & Aggleton, J. P. (2004). Anterior thalamic lesions stop immediate early gene activation in selective laminae of the retrosplenial cortex: evidence of covert pathology in rats? *European Journal of Neuroscience, 19*(12), 3291-3304.

Jo, Y. S., & Lee, I. (2010). Disconnection of the hippocampal–perirhinal cortical circuits severely disrupts object–place paired associative memory. *Journal of Neuroscience, 30*(29), 9850-9858.

Jones, B. F., & Witter, M. P. (2007). Cingulate cortex projections to the parahippocampal region and hippocampal formation in the rat. *Hippocampus, 17*(10), 957-976.

Jones, D. T., Mateen, F. J., Lucchinetti, C. F., Jack, C. R., & Welker, K. M. (2011). Default mode network disruption secondary to a lesion in the anterior thalamus. *Archives of Neurology, 68*(2), 242-247.

Jones, M. W., Errington, M. L., French, P. J., Fine, A., Bliss, T. V. P., Garel, S., . . . Davis, S. (2001). A requirement for the immediate early gene Zif268 in the expression of late LTP and long-term memories. *Nature Neuroscience, 4*(3), 289.

Jones, M. W., French, P. J., Bliss, T. V. P., & Rosenblum, K. (1999). Molecular mechanisms of long-term potentiation in the insular cortex in vivo. *Journal of Neuroscience, 19*(21), RC36-RC36.

Kart-Teke, E., Silva, M. A. D. S., Huston, J. P., & Dere, E. (2006). Wistar rats show episodic-like memory for unique experiences. *Neurobiology of Learning and Memory, 85*(2), 173-182.

Kehoe, E. J. (1988). A layered network model of associative learning: learning to learn and configuration. *Psychological review, 95*(4), 411.

Kesner, R. P., Gilbert, P. E., & Barua, L. A. (2002). The role of the hippocampus in memory for the temporal order of a sequence of odors. *Behavioral Neuroscience, 116*(2), 286.

Kesner, R. P., Hunsaker, M. R., & Gilbert, P. E. (2005). The role of CA1 in the acquisition of an object-trace-odor paired associate task. *Behavioral Neuroscience, 119*(3), 781.

Kinnavane, L., Amin, E., Aggleton, J. P., & Nelson, A. J. D. (2019). Do the rat anterior thalamic nuclei contribute to behavioural flexibility? *Behavioural Brain Research, 359*, 536-549.

Knapska, E., & Kaczmarek, L. (2004). A gene for neuronal plasticity in the mammalian brain: Zif268/Egr-1/NGFI-A/Krox-24/TIS8/ZENK? *PROGRESS IN NEUROBIOLOGY, 74*(4), 183-211.

Kopelman, M. D. (2015). What does a comparison of the alcoholic Korsakoff syndrome and thalamic infarction tell us about thalamic amnesia? *Neuroscience & Biobehavioral Reviews, 54*, 46-56.

Kopelman, M. D., Thomson, A. D., Guerrini, I., & Marshall, E. J. (2009). The Korsakoff Syndrome: Clinical Aspects, Psychology and Treatment. *Alcohol & Alcoholism, 44*(2), 148-154. https://doi.org/10.1093/alcalc/agn118

Kubik, S., Miyashita, T., & Guzowski, J. F. (2007). Using immediate-early genes to map hippocampal subregional functions. *Learning & Memory, 14*(11), 758-770.

Langston, R. F., Stevenson, C. H., Wilson, C. L., Saunders, I., & Wood, E. R. (2010). The role of hippocampal subregions in memory for stimulus associations. *Behavioural Brain Research, 215*(2), 275-291.

Langston, R. F., & Wood, E. R. (2009). Associative recognition and the hippocampus: Differential effects of hippocampal lesions on object-place, object-context and object-place-context memory. *Hippocampus, 20*(10), 1139-1153.

Ledoux, J. (2002). *Synaptic Self*. New York: Viking.

Lee, I., & Solivan, F. (2008). The roles of the medial prefrontal cortex and hippocampus in a spatial paired-association task. *Learning & Memory, 15*(5), 357-367.

Li, S., Kumar, Y., Gupta, N., Abdelbaki, A., Sahwney, H., Kumar, A., . . . Mangla, R. (2018). Clinical and neuroimaging findings in thalamic territory infarctions: a review. *Journal of Neuroimaging, 28*(4), 343-349.

Lopez, J., Wolff, M., Lecourtier, L., Cosquer, B., Bontempi, B., Dalrymple-Alford, J. C., & Cassel, J. C. (2009). The Intralaminar Thalamic Nuclei Contribute to Remote Spatial Memory. *Journal of Neuroscience, 29*(10), 3302-3306. https://doi.org/10.1523/JNEUROSCI.5576-08.2009

Loukavenko, E. A., Ottley, M. C., Moran, J. P., Wolff, M., & Dalrymple-Alford, J. C. (2007). Towards therapy to relieve memory impairment after anterior thalamic lesions: improved spatial working memory after immediate and delayed postoperative enrichment. *The European journal of neuroscience, 26*(11), 3267-3276. https://doi.org/10.1111/j.1460-9568.2007.05879.x

Loukavenko, E. A., Wolff, M., Poirier, G. L., & Dalrymple-Alford, J. C. (2015). Impaired spatial working memory after anterior thalamic lesions: recovery with cerebrolysin and enrichment. *Brain Structure and Function*. https://doi.org/10.1007/s00429-015-1015-x

Magdalena, P., Medina, J. H., & Cynthia, K. (2020). Anterior retrosplenial cortex is required for long-term object recognition memory. *Scientific Reports (Nature Publisher Group), 10*(1).

Mair, R. G., Burk, J. A., & Porter, M. C. (2003). Impairment of Radial Maze Delayed Nonmatching After Lesions of Anterior Thalamus and Parahippocampal Cortex. *Behavioral Neuroscience, 117*(3), 596-605. https://doi.org/10.1037/0735-7044.117.3.596

Mair, W. G., Warrington, E. K., & Weiskrantz, L. (1979). Memory disorder in Korsakoff's psychosis: a neuropathological and neuropsychological investigation of two cases. *Brain: a journal of neurology, 102*(4), 749-783.

Manns, J. R., & Eichenbaum, H. B. (2005). Time and treason to the trisynaptic teachings: theoretical comment on Kesner et Al.(2005).

Mathiasen, M. L., Dillingham, C. M., Kinnavane, L., Powell, A. L., & Aggleton, J. P. (2017, 5/4/). Asymmetric cross-hemispheric connections link the rat anterior thalamic nuclei with the cortex and hippocampal formation. *Neuroscience, 349*, 128-143. https://doi.org/https://doi.org/10.1016/j.neuroscience.2017.02.026

Maviel, T., Durkin, T. P., Menzaghi, F., & Bontempi, B. (2004). Sites of neocortical reorganization critical for remote spatial memory. *Science, 305*(5680), 96-99.

Mayes, A. R., Meudell, P. R., Mann, D., & Pickering, A. (1988). Location of lesions in Korsakoff's syndrome: neuropsychological and neuropathological data on two patients. *Cortex, 24*(3), 367-388.

McClelland, J. L., McNaughton, B. L., & O'Reilly, R. C. (1995). Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. *Psychological review, 102*(3), 419.

Méndez-Couz, M., Conejo, N. M., Vallejo, G., & Arias, J. L. (2014). Spatial memory extinction: A c-Fos protein mapping study. *Behavioural Brain Research, 260*, 101-110.

Mendez-Lopez, M., Arias, J. L., Bontempi, B., & Wolff, M. (2013). Reduced cytochrome oxidase activity in the retrosplenial cortex after lesions to the anterior thalamic nuclei. *Behavioural Brain Research, 250*, 264-273.

Mitchell, A. S., & Dalrymple-Alford, J. C. (2005). Dissociable memory effects after medial thalamus lesions in the rat. *The European journal of neuroscience, 22*(4), 973-985. https://doi.org/10.1111/j.1460-9568.2005.04199.x

Mitchell, A. S., & Dalrymple-Alford, J. C. (2006). Lateral and anterior thalamic lesions impair independent memory systems. *Learning & memory (Cold Spring Harbor, N.Y.), 13*(3), 388-396. https://doi.org/10.1101/lm.122206

Mitchell, A. S., Dalrymple-Alford, J. C., & Christie, M. A. (2002). Spatial Working Memory and the Brainstem Cholinergic Innervation to the Anterior Thalamus. *Journal of Neuroscience, 22*(5), 1922-1928.

Moran, J. P., & Dalrymple-Alford, J. C. (2003). Perirhinal Cortex and Anterior Thalamic Lesions: Comparative Effects on Learning and Memory. *Behavioral Neuroscience, 117*(6), 1326. https://doi.org/10.1037/0735-7044.117.6.1326

Moreau, P. H., Tsenkina, Y., Lecourtier, L., Lopez, J., Cosquer, B., Wolff, M., . . . Cassel, J. C. (2013). Lesions of the anterior thalamic nuclei and intralaminar thalamic nuclei: place and visual discrimination learning in the water maze. *Brain structure & function, 218*(3), 657-667. https://doi.org/10.1007/s00429-012-0419-0

Morris, R. G. M. (1983). An attempt to dissociate" spatial-mapping and working-memory" theories of hippocampal function. *Neurobiology of the hippocampus*, 405-432.

Nadel, L., & Moscovitch, M. (1997). Memory consolidation, retrograde amnesia and the hippocampal complex. *Current Opinion in Neurobiology, 7*(2), 217-227.

Olton, D. S., Becker, J. T., & Handelmann, G. E. (1979). Hippocampus, space, and memory. *Behavioral and Brain Sciences, 2*(3), 313-322.

Papez, J. W. (1937). A proposed mechanism of emotion. *Archives of Neurology & Psychiatry, 38*(4), 725-743.

Paxinos, G., & Watson, C. (1998). *The rat brain in stereotaxic coordinates*. San Diego: Academic Press.

Paxinos, G., & Watson, C. (2014). *Paxinos and Watson's The Rat Brain in Stereotaxic Coordinates, 7th Edition.*: Elsevier Academic Press, San Diego.

Perry, B. A. L., Mercer, S. A., Barnett, S. C., Lee, J., & Dalrymple-Alford, J. C. (2018). Anterior thalamic nuclei lesions have a greater impact than mammillothalamic tract lesions on the extended hippocampal system. *Hippocampus, 28*(2), 121-135.

Pitel, A. L., Chételat, G., Le Berre, A. P., Desgranges, B., Eustache, F., & Beaunieux, H. (2012). Macrostructural abnormalities in Korsakoff syndrome compared with uncomplicated alcoholism. *Neurology, 78*(17), 1330-1333.

Poirier, G. L., & Aggleton, J. P. (2009). Post-surgical interval and lesion location within the limbic thalamus determine extent of retrosplenial cortex immediate-early gene hypoactivity. *Neuroscience, 160*(2), 452-469.

Poirier, G. L., Shires, K. L., Sugden, D., Amin, E., Thomas, K. L., Carter, D. A., & Aggleton, J. P. (2008). Anterior thalamic lesions produce chronic and profuse transcriptional deregulation in retrosplenial cortex: a model of retrosplenial hypoactivity and covert pathology. *Thalamus & Related Systems, 4*(1), 59-77.

Powell, A. L., Vann, S. D., Olarte-Sánchez, C. M., Kinnavane, L., Davies, M., Amin, E., . . . Nelson, A. J. D. (2017). The retrosplenial cortex and object recency memory in the rat. *European Journal of Neuroscience, 45*(11), 1451-1464.

Preston, A. R., & Eichenbaum, H. B. (2013). Interplay of hippocampus and prefrontal cortex in memory. *Current Biology, 23*(17), R764-R773.

Price, J. L. (1995). Thalamus. *The rat nervous system, 2*, 629-648.

Ranganath, C. (2010). A unified framework for the functional organization of the medial temporal lobes and the phenomenology of episodic memory. *Hippocampus, 20*(11), 1263-1290.

Rawlins, J. N. P. (1985). Associations across time: The hippocampus as a temporary memory store. *Behavioral and Brain Sciences, 8*(3), 479-497.

Rocca, M. A., Amato, M. P., De Stefano, N., Enzinger, C., Geurts, J. J., Penner, I.-K., . . . Filippi, M. (2015). Clinical and imaging assessment of cognitive dysfunction in multiple sclerosis. *The Lancet Neurology, 14*(3), 302-317.

Rogers, J. L., & Kesner, R. P. (2007). Hippocampal–parietal cortex interactions: evidence from a disconnection study in the rat. *Behavioural Brain Research, 179*(1), 19-27.

Rüb, U., Del Tredici, K., Schultz, C., Ghebremedhin, E., De Vos, R. A. I., Steur, E. J., & Braak, H. (2002). Parkinson's disease: the thalamic components of the limbic loop are severely impaired by α-synuclein immunopositive inclusion body pathology. *Neurobiology of aging, 23*(2), 245-254.

Schacter, D. L., & Addis, D. R. (2009). On the nature of medial temporal lobe contributions to the constructive simulation of future events. *Philosophical Transactions of the Royal Society B: Biological Sciences, 364*(1521), 1245-1253.

Schmahmann, J. D. (2003). Vascular syndromes of the thalamus. *Stroke, 34*(9), 2264-2278.

Sechi, G., & Serra, A. (2007). Wernicke's encephalopathy: new clinical settings and recent advances in diagnosis and management. *The Lancet Neurology, 6*(5), 442-455.

Segobin, S., Laniepce, A., Ritz, L., Lannuzel, C., Boudehent, C., Cabé, N., . . . Beaunieux, H. (2019). Dissociating thalamic alterations in alcohol use disorder defines specificity of Korsakoff's syndrome. *Brain, 142*(5), 1458-1470.

Shibata, H. (1992). Topographic organization of subcortical projections to the anterior thalamic nuclei in the rat. *Journal of Comparative Neurology, 323*(1), 117-127.

Shibata, H. (1993). Efferent projections from the anterior thalamic nuclei to the cingulate cortex in the rat. *Journal of Comparative Neurology, 330*(4), 533-542.

Shibata, H., & Kato, A. (1993). Topographic relationship between anteromedial thalamic nucleus neurons and their cortical terminal fields in the rat. *Neuroscience Research, 17*(1), 63-69.

Shibata, H., & Naito, J. (2005). Organization of anterior cingulate and frontal cortical projections to the anterior and laterodorsal thalamic nuclei in the rat. *Brain Research, 1059*(1), 93-103.

Shimamura, A. P., Jernigan, T. L., & Squire, L. R. (1988). Korsakoff's syndrome: Radiological (CT) findings and neuropsychological correlates. *Journal of Neuroscience, 8*(11), 4400-4410.

Sicotte, N. L., Kern, K. C., Giesser, B. S., Arshanapalli, A., Schultz, A., Montag, M., . . . Bookheimer, S. Y. (2008). Regional hippocampal atrophy in multiple sclerosis. *Brain, 131*(4), 1134-1141.

Squire, L. R. (1992). Declarative and nondeclarative memory: Multiple brain systems supporting learning and memory. *Journal of cognitive neuroscience, 4*(3), 232-243.

Squire, L. R., Amaral, D. G., Zola-Morgan, S., Kritchevsky, M., & Press, G. (1989). Description of brain injury in the amnesic patient NA based on magnetic resonance imaging. *Experimental neurology, 105*(1), 23-35.

Squire, L. R., & Kandel, E. R. (2003). *Memory: From mind to molecules* (Vol. 69): Macmillan.

Squire, L. R., Stark, C. E. L., & Clark, R. E. (2004). The medial temporal lobe. *Annu. Rev. Neurosci., 27*, 279-306.

Squire, L. R., & Wixted, J. T. (2011). The cognitive neuroscience of human memory since HM. *Annual Review of Neuroscience, 34*, 259-288.

St-Laurent, M., Petrides, M., & Sziklas, V. (2009). Does the cingulate cortex contribute to spatial conditional associative learning in the rat? *Hippocampus, 19*(7), 612-622.

Sumowski, J. F., Benedict, R. H. B., Enzinger, C., Filippi, M., Geurts, J. J., Hamalainen, P., . . . Rocca, M. A. (2018). Cognition in multiple sclerosis: State of the field and priorities for the future. *Neurology, 90*(6), 278-288.

Sziklas, V., & Petrides, M. (1999). The effects of lesions to the anterior thalamic nuclei on object-place associations in rats. *The European journal of neuroscience, 11*(2), 559-566. https://doi.org/10.1046/j.1460-9568.1999.00448.x

Sziklas, V., & Petrides, M. (2002). Effects of lesions to the hippocampus or the fornix on allocentric conditional associative learning in rats. *Hippocampus, 12*(4), 543-550.

Sziklas, V., & Petrides, M. (2004). Egocentric conditional associative learning: Effects of restricted lesions to the hippocampo-mammillo-thalamic pathway. *Hippocampus, 14*(8), 931-934. https://doi.org/10.1002/hipo.20044

Sziklas, V., & Petrides, M. (2007). Contribution of the anterior thalamic nuclei to conditional learning in rats. *Hippocampus, 17*(6), 456-461. https://doi.org/10.1002/hipo.20286

Tischmeyer, W., & Grimm, R. (1999). Activation of immediate early genes and memory formation. *Cellular and Molecular Life Sciences CMLS, 55*(4), 564-574.

Tsivilis, D., Vann, S. D., Denby, C., Roberts, N., Mayes, A. R., Montaldi, D., & Aggleton, J. P. (2008). A disproportionate role for the fornix and mammillary bodies in recall versus recognition memory. *Nature Neuroscience, 11*(7), 834-842. https://doi.org/10.1038/nn.2149

Tulving, E. (2002). Episodic memory: From mind to brain. *Annual review of psychology, 53*(1), 1-25.

Ulrich, K., Aitken, P. N., Abraham, W. C., Dalrymple-Alford, J. C., & McNaughton, N. (2014). Effects of thalamic lesions on repeated relearning of a spatial working memory task. *Behavioural Brain Research, 261*, 56.

Van Der Werf, Y. D., Scheltens, P., Lindeboom, J., Witter, M. P., Uylings, H. B. M., & Jolles, J. (2003). Deficits of memory, executive functioning and attention following infarction in the thalamus; a study of 22 cases with localised lesions. *Neuropsychologia, 41*(10), 1330-1344.

van Groen, T., Kadish, I., & Wyss, J. M. (2002). Role of the anterodorsal and anteroventral nuclei of the thalamus in spatial memory in the rat. *Behavioural Brain Research, 132*(1), 19-28. https://doi.org/10.1016/S0166-4328(01)00390-4

van Groen, T., & Wyss, J. M. (1990a). The connections of presubiculum and parasubiculum in the rat. *Brain Research, 518*(1-2), 227-243.

van Groen, T., & Wyss, J. M. (1990b). Connections of the retrosplenial granular a cortex in the rat. *The Journal of comparative neurology, 300*(4), 593-606. https://doi.org/10.1002/cne.903000412

van Groen, T., & Wyss, J. M. (1992). Connections of the retrosplenial dysgranular cortex in the rat. *Journal of Comparative Neurology, 315*(2), 200-216.

van Groen, T., & Wyss, J. M. (2003). Connections of the retrosplenial granular b cortex in the rat. *Journal of Comparative Neurology, 463*(3), 249-263.

Vann, S. D. (2013). Dismantling the Papez circuit for memory in rats. *ELIFE, 2*(2), e00736. https://doi.org/10.7554/eLife.00736

Vann, S. D., & Aggleton, J. P. (2004). The mammillary bodies: two memory systems in one? *Nature Reviews Neuroscience, 5*(1), 35-44. https://doi.org/10.1038/nrn1299

Vann, S. D., Erichsen, J. T., O'Mara, S. M., & Aggleton, J. P. (2011). Selective disconnection of the hippocampal formation projections to the mammillary bodies produces only mild deficits on spatial memory tasks: implications for fornix function. *Hippocampus, 21*(9), 945-957.

Vann, S. D., Saunders, R. C., & Aggleton, J. P. (2007). Distinct, parallel pathways link the medial mammillary bodies to the anterior thalamus in macaque monkeys. *The European journal of neuroscience, 26*(6), 1575-1586. https://doi.org/10.1111/j.1460-9568.2007.05773.x

Victor, M., Adams, R. D., & Collins, G. H. (1971). The Wernicke-Korsakoff syndrome. A clinical and pathological study of 245 patients, 82 with post-mortem examinations. *Contemporary neurology series, 7*, 1.

Vogt, B. A., & Paxinos, G. (2014). Cytoarchitecture of mouse and rat cingulate cortex with human homologies. *Brain Structure and Function, 219*(1), 185-192.

Warburton, E. C., & Aggleton, J. P. (1999). Differential deficits in the Morris water maze following cytotoxic lesions of the anterior thalamus and fornix transection. *Behavioural Brain Research, 98*(1), 27-38.

Warburton, E. C., Baird, A. L., Morgan, A., Muir, J. L., & Aggleton, J. P. (2000). Disconnecting hippocampal projections to the anterior thalamus produces deficits on tests of spatial memory in rats. *The European journal of neuroscience, 12*(5), 1714-1726. https://doi.org/10.1046/j.1460-9568.2000.00039.x

Warburton, E. C., Baird, A. L., Morgan, A., Muir, J. L., & Aggleton, J. P. (2001). The conjoint importance of the hippocampus and anterior thalamic nuclei for allocentric spatial learning: evidence from a disconnection study in the rat. *Journal of Neuroscience, 21*(18), 7323-7330.

Warburton, E. C., & Brown, M. W. (2015). Neural circuitry for rat recognition memory. *Behavioural Brain Research*. https://doi.org/10.1016/j.bbr.2014.09.050

Warburton, E. C., Morgan, A., Baird, A. L., Muir, J. L., & Aggleton, J. P. (1999). Does Pretraining Spare the Spatial Deficit Associated With Anterior Thalamic Damage in Rats? *Behavioral Neuroscience, 113*(5), 956-967. https://doi.org/10.1037/0735-7044.113.5.956

Ward-Robinson, J., Wilton, L. A. K., Muir, J. L., Honey, R. C., Vann, S. D., & Aggleton, J. P. (2002). Sensory preconditioning in rats with lesions of the anterior thalamic nuclei: evidence for intact nonspatial 'relational' processing. *Behavioural Brain Research, 133*(2), 125-133. https://doi.org/10.1016/S0166-4328(01)00465-X

Wernicke, C. (1881). *Lehrbuch der gehirnkrankheiten für aerzte und studirende* (Vol. 2): Fischer.

Wolff, M., Gibb, S. J., Cassel, J. C., & Dalrymple-Alford, J. C. (2008). Anterior but not intralaminar thalamic nuclei support allocentric spatial memory. *Neurobiology of Learning and Memory, 90*(1), 71-80. https://doi.org/10.1016/j.nlm.2008.01.007

Wolff, M., Gibb, S. J., & Dalrymple-Alford, J. C. (2006). Beyond Spatial Memory: The Anterior Thalamus and Memory for the Temporal Order of a Sequence of Odor Cues. *Journal of Neuroscience, 26*(11), 2907-2913. https://doi.org/10.1523/jneurosci.5481-05.2006

Wolff, M., Loukavenko, E. A., Will, B. E., & Dalrymple-Alford, J. C. (2008). The extended hippocampal-diencephalic memory system: enriched housing promotes recovery of the flexible use of spatial representations after anterior thalamic lesions. *Hippocampus, 18*(10), 996-1007. https://doi.org/10.1002/hipo.20457

Wong-Riley, M. T. T. (1989). Cytochrome oxidase: an endogenous metabolic marker for neuronal activity. *Trends in neurosciences, 12*(3), 94-101.

Wright, N. F., Erichsen, J. T., Vann, S. D., O'Mara, S. M., & Aggleton, J. P. (2010). Parallel but separate inputs from limbic cortices to the mammillary bodies and anterior thalamic nuclei in the rat. *Journal of Comparative Neurology, 518*(12), 2334-2354.

Wright, N. F., Vann, S. D., Aggleton, J. P., & Nelson, A. J. D. (2015). A Critical Role for the Anterior Thalamus in Directing Attention to Task-Relevant Stimuli. *The Journal of Neuroscience, 35*(14), 5480-5488.

Wyss, J. M., & Van Groen, T. (1992). Connections between the retrosplenial cortex and the hippocampal formation in the rat: a review. *Hippocampus, 2*(1), 1-11.